

'Kasalath' Allele in Nipponbare Background is Responsible for the Plasticity in Lateral Root Development of Rice under Soil Moisture Fluctuation Stress

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Soil moisture fluctuations (SMF) from wet to dry and vice-versa are common under field conditions, which influence root growth and functions and thus dry matter production and yield. In such condition, phenotypic plasticity in L-type lateral root (LLR) development is an adaptive mechanism of rice and the associated quantitative trait locus (QTL) was previously detected in chromosome 12, mainly contributed by the 'Kasalath' allele in Nipponbare x Kasalath chromosome segment substitution lines (CSSLs). In this study, we used +qLLRN-12 genotype of Nipponbare background to validate the functions for LR plasticity under SMF and progressive drought. Plants were subjected to well-watered, fluctuating soil moisture and progressive drought conditions for 38 d. There were no significant genotypic differences in shoot growth and root development under well-watered condition. On the other hand, +qLLRN-12 genotype showed greater shoot dry weight by 31% than Nipponbare, which was associated with larger root system of the former than the latter genotype under fluctuating soil moistures. The greater root system development of +qLLRN-12 genotype was attributed to the greater L-type LR development by 95% relative to Nipponbare. However, under progressive drought condition, +qLLRN-12 genotype had reduced shoot dry weight (SDW) due to its smaller root system relative to its fluctuating soil moisture and well-watered counterparts. These results indicate that the introgressed segment of Kasalath on the chromosome 12 region of Nipponbare was responsible for the plasticity in L-type LR, which contributed to greater root system development, increased water uptake and consequently increased dry matter production under fluctuating soil moisture conditions. The findings also suggest that the expression of this allele is unique and triggered only under fluctuating moisture stress conditions.

Key Words: chromosome segment substitution lines (CSSLs), lateral root plasticity, phenotypic plasticity, quantitative trait locus, root system development, soil moisture fluctuation stress

Abbreviations: C-D – continuous drought, CSSLs – chromosome segment substitution lines, D-W – transient drought-to-waterlogged, LR – lateral root, NR – nodal root, QTL – quantitative trait loci, RDW – root dry weight, RSR – root-to-shoot ratio, SDW – shoot dry weight, SMF – small moisture fluctuation, TRL – total root length, W-D – transient waterlogged-to-drought, WW – well-watered

INTRODUCTION

In rainfed lowland rice ecosystem, rainfall is erratic and unpredictable, and standing water disappears at any time in the growing season, resulting in various intensities of soil moisture stress which significantly limit shoot growth and root development and, eventually, reduce grain yield. In rice, deficit (drought) or excess (submergence) of

water negatively affects productivity. Thus, the inability of plants to acclimate to the above conditions would lead to reductions in dry matter and grain yield. Roots are the forefront and key in adaptation to any water stresses. Because roots serve as interfaces between plant and the soil, they are primarily and more highly affected by drought stress compared with the aerial parts of the plant (Franco et al. 2011).

Root systems play a key role in enhancing crop yield and adaptation in response to various environments (Wang and Yamauchi 2006; Yamauchi et al. 1996). Active and responsive root systems for greater adaptation to water stresses have a significant role in maintaining greater water and nutrient uptake from the soil, and consequently, crop growth and yield.

Several studies have shown that variability in soil moisture conditions adversely affects shoot and root growth and functions of 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) as well as soil nutrient availability (Kondo et al. 2005; Iijima et al. 2007; Suralta 2010).

We have demonstrated that root plasticity has a significant functional role in plant adaptation under progressive drought and soil moisture fluctuation (SMF) stresses (Yamauchi et al. 1996; Wang and Yamauchi 2006; Kano-Nakata et al. 2011; Suralta et al. 2010; Niones et al. 2012). Root plasticity is defined as the ability of a plant to developmentally alter (promote) its root phenotype and to function in response to changing environmental conditions as has been suggested (O'Toole and Bland 1987) and validated quantitatively (Wang and Yamauchi 2006).

In rice, genotypic variations in the plasticity of root traits such as lateral roots have been reported under 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 waterlogging and drought (Niones et al. 2012). The lateral root (LR) generally comprises the greater proportion of the whole root system and thus, its response to moisture stress directly reflects the performance of the entire root system (Yamauchi et al. 1987; Wang et al. 2009; Niones et al. 2012). Lateral roots are classified into the L-type and S-type lateral roots (Yamauchi et al. 1996). Moreover, the L-type LR is a long and branching type lateral root while the S-type LR is a short and non-branching type of lateral root.

This LR plasticity, especially the L-type LR, significantly contributes to maintaining root system development for better adaptation to drought conditions (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). Enhanced LR development increases root surface area and soil water extraction

(Kamoshita et al. 2000, 2004; Siopongco et al. 2005, 2006; Kato et al. 2011; Henry et al. 2011), and water use (Kobata et al. 1996; Suralta et al. 2010).

Using the selected chromosome segment substitution line (CSSL) from Nipponbare x Kasalath crosses such as CSSL47, we have quantified precisely the functional roles of root plasticity (i.e., lateral root plasticity under SMF) in the maintenance of dry matter production and grain yield (Suralta et al. 2010; Niones et al. 2012). We assumed that genetically, one or more substituted segments from the Kasalath allele introgressed into the Nipponbare genetic background in CSSL47 regulate LR plasticity. We have identified quantitative trait loci (QTL), designated as *+qLLRN-12*, associated with the plasticity in LR on the short-arm of chromosome 12, with increased effect from Kasalath alleles particularly expressed under SMF during the seedling and vegetative stages (Niones et al. 2015). The plasticity in LR is essential for the development and establishment of root system structure during SMF occurrence both at the seedling and vegetative stages, which would increase root surface area, and thus, increase water and nutrient absorption (Niones et al. 2012). Furthermore, development of more LRs in the root system at the early stages of growth significantly contributed to the increase in total dry matter production under SMF (Niones et al. 2012). Several studies have shown that the plasticity in LR production contributed to higher photosynthesis, stomatal conductance and transpiration, thus resulting in increased shoot dry matter production in rice (Kano et al. 2011; Kano-Nakata et al. 2011; Bañoc et al. 2000; Suralta et al. 2010; Siopongco et al. 2006). Under SMF, function and expression of this specific substituted segment from Kasalath allele on chromosome 12 needs further confirmation. Hence, this study aimed to (1) validate the functions of the QTL associated with L-type LR plasticity using CSSL52 under SMF and (2) examine if this Kasalath allele on chromosome 12 regions has functional contribution under progressive drought condition stress.

MATERIALS AND METHODS

Plant Materials

Three rice genotypes, lowland adapted *japonica* Nipponbare and two CSSL lines (CSSL47 and CSSL52), were used in the study. Nipponbare is a recurrent parent used for the development of the 54 CSSL (Nipponbare/Kasalath crosses) population by the Rice Genome Research Center of the National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan. Among CSSLs, one line (CSSL47) was selected due to its unique characteristics of root system development, particularly the promoted LR development under SMF conditions (Suralta et al. 2010;

Niones et al. 2012). However, this CSSL47 carries substituted segments from the Kasalath allele in ten chromosomes of Nipponbare genetic background (RGP 2000; RGP 2010). On the other hand, CSSL52 (referred to as *+qLLRN-12* genotype) was selected because it carries only two substituted segments from the Kasalath allele in the Nipponbare genetic background. These substituted segments are found at chromosome 2 (R1843 loci), and chromosome 12 (between G24B and C443 locus). The substituted segment from the Kasalath allele at the chromosome 12 region is the same as that found in CSSL47 and is the major point of interest in this study. CSSL52 has fewer introgressed segment of the Kasalath allele in other chromosomes than CSSL47 (RGP 2000; RGP 2010), making it ideal to use in the validation of, and is therefore more ideal in validating precisely the QTL associated with LR plasticity.

Phenotyping and Soil Moisture Treatments

A greenhouse study was conducted at the experimental field of Nagoya University, Nagoya, Aichi Prefecture, Japan (136°56'6"E, 35°9'5"N). Seeds of each genotype were soaked in water containing benomyl fungicide (0.15% w/v) for 30 min, washed thoroughly and then incubated in a seed germinator at 28°C for 36 h. Two pre-germinated seeds from each genotype were grown in PVC boxes (25 cm × 2 cm × 40 cm, *L × W × H*) filled with 2.5 kg of air-dried sandy loam soil following the method of Suralta et al. (2008a). The soil in each box was pre-mixed with complete fertilizer containing 60 mg nitrogen (N), 80 mg phosphorus (P), and 70 mg potassium (K). The seedlings were thinned to one seedling per box at 3 d after sowing (DAS). All genotypes were exposed to three soil moisture contents (SMC): well-watered (WW) control, soil moisture fluctuation (SMF) and continuous drought (C-D) conditions. SMF was applied by transient waterlogged-to-drought (W-D) and transient drought-to-waterlogged (D-W). Furthermore, in addition to the above soil moisture treatments, *+qLLRN-12* was also subjected to C-D condition. The Nipponbare and CSSL47 were excluded and not exposed to C-D conditions because in our previous studies, CSSL47 reduction of shoot dry matter production and root growth were comparable with Nipponbare in response to progressive drought stress (Niones et al. 2015; Kano et al. 2011), thus we assumed the response of the said genotypes would be the same under this condition.

In WW which served as the control, the water level was maintained at the soil surface of the root box. In the SMF treatment, application and exposure were as follows: In the W-D treatment, plants were subjected to waterlogging for 17 d and then to drought for 21 d while

in the D-W treatments, plants were subjected to drought stress for 17 d, and then to waterlogging conditions for another 21 d. Under C-D conditions, the *+qLLRN-12* plants were exposed to continuous drought stress from sowing to 38 d after sowing (DAS). Target soil moisture content (SMC) under C-D condition was maintained at 20% (w/w) by replacing the amount of water lost every day. Plant sampling was done at 38 DAS with four boxes (1 box = 1 replication) for each treatment.

Shoot and Root Growth Measurements

Shoots were cut and placed in a paper bag and oven dried at 70°C for 48 h and weighed.

Root sampling was carried out following the methods of Kono et al. (1987) and Suralta et al. (2010). Briefly, 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 h. 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; 2,592 × 3872 resolution). Cleaned root samples were then stored in FAA (formalin: acetic acid: 70% ethanol in 1:1:18 ratio by volume) solution for other measurements.

The total number of nodal root and LR was manually counted. Each coleoptile nodal root was cut into 5-cm segments, keeping the LRs intact. The number of each type of LR in each of the 5-cm coleoptile nodal root segment was manually counted. The number of LRs was then expressed as linear frequency along NR (number of LRs per unit length of nodal root axis, Ito et al. 2006). The length of each nodal root was manually measured using a ruler. For 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 WinRhizo software version 2009 (Regent Instruments Canada, Inc.).

Statistical Analysis

The experiments were laid out in a randomized complete block design with four replications (1 box = 1 replication). Treatment means were compared using the least significant difference (LSD) test at *P*<0.05 level of significance.

RESULTS

Shoot Growth

The SDW of *+qLLRN-12* (2.50 g/plant) and CSSL47 (2.45 g/

plant) genotypes were not significantly different from that of Nipponbare (2.40 g/plant) under WW conditions (Fig. 1). Under both W-D and D-W of SMF conditions, SDW was significantly reduced in CSSL47 and Nipponbare but not in +qLLRN-12 relative to WW conditions (Fig. 1). The SDW of +qLLRN-12 (2.50 g/plant) genotype was significantly greater than that of Nipponbare (1.91 g/plant) under SMF conditions. Furthermore, +qLLRN-12 genotype had significantly reduced SDW by 32% relative to WW condition but was not significantly affected under D-W and W-D conditions (Table 1).

Root System Development

The root system profiles of the three genotypes under different soil moisture treatments are shown in Fig. 2. Evidently, the +qLLRN-12 and CSSL47 genotypes developed larger root system than Nipponbare in response to SMF conditions (Fig. 2e, f, h and i). Under WW condition, TRL, number of LR, and NR were not significantly different among genotypes (Table 1). On the other hand, significant genotypic differences in TRL, LR and NR were observed in response to SMF conditions. The +qLLRN-12 and CSSL47 genotypes had longer total root length than Nipponbare by 10% or more under both D-W and W-D. The number of L-type LRs was also significantly higher in +qLLRN-12 and CSSL47 by 30–38% under D-W and by 54–169% under W-D, than in Nipponbare (Table 1). Between the two SMF conditions, L-type LRs were generally more promoted across genotypes under W-D (150%) than D-W (78%) conditions relative to their WW counterparts (Table 1).

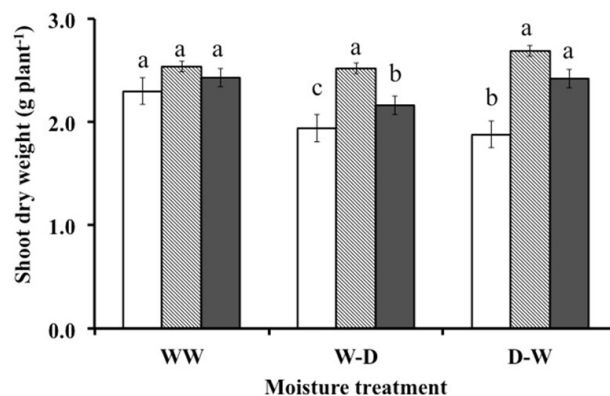


Fig. 1. Shoot dry weight of a 38-d-old Nipponbare (□), +qLLRN-12 (▨) and CSSL47 (■) under well-watered (WW), transient waterlogging-to-drought (W-D) and transient drought-to-waterlogging (D-W) conditions for 38 d. Bars followed by the same letter within each water treatment are not significantly different at $P < 0.05$. Data are means of 3 replicates \pm standard error.

The number of S-type LRs was generally similar among genotypes under SMF conditions except in W-D where +qLLRN-12 had significantly lower S-type LRs than Nipponbare by 35%. Accordingly, the total number of LRs was significantly higher in both +qLLRN-12 and CSSL47 than in Nipponbare by 14–20% under D-W and 6–12% under W-D conditions. The total length of lateral roots was also significantly higher in both +qLLRN-12 and CSSL47 than in Nipponbare by 9–19% under D-W and 7–20% under W-D conditions.

The number of NR of +qLLRN-12 and CSSL47

Table 1. Root system development of a 38-d-old Nipponbare, CSSL47 and +qLLRN-12 genotypes grown under well-watered (WW), transient drought-to-waterlogging (D-W) and transient waterlogging-to-drought (W-D) conditions.

Soil Moisture Treatment	Genotypes	Total Root (m/ plant)	Lateral Roots (LR)			Nodal Roots (NR)			
			(number LR cm ⁻¹)			Length (m/ plant)	Number (no./ plant)	Length (cm/ plant)	Total Length (m/plant)
			L-type	S-type	Total				
WW	Nipponbare	9.9 ^a	0.9 ^a	4.5 ^a	5.4 ^a	5.9 ^a	93.0 ^a	43.2 ^a	4.0 ^a
	+qLLRN-12	10.2 ^a	0.9 ^a	4.6 ^a	5.4 ^a	5.8 ^a	106.3 ^a	41.7 ^a	4.4 ^a
	CSSL47	10.0 ^a	0.9 ^a	5.1 ^a	6.0 ^a	5.9 ^a	110.3 ^a	36.8 ^a	4.1 ^a
D-W	Nipponbare	10.2 ^b	1.3 ^b	4.5 ^a	5.4 ^b	6.8 ^b	69.3 ^b	48.3 ^a	3.4 ^b
	+qLLRN-12	11.4 ^a	1.7 ^a	4.6 ^a	6.2 ^a	7.4 ^a	102.0 ^a	39.0 ^b	4.0 ^a
	CSSL47	12.0 ^a	1.8 ^a	4.7 ^a	6.5 ^a	8.1 ^a	84.7 ^a	45.7 ^a	3.9 ^a
W-D	Nipponbare	7.1 ^b	1.3 ^c	5.2 ^a	6.5 ^c	4.4 ^b	69.3 ^b	39.2 ^b	2.7 ^b
	+qLLRN-12	7.9 ^a	3.5 ^a	3.4 ^b	6.9 ^b	5.3 ^a	67.3 ^b	38.8 ^b	2.6 ^b
	CSSL47	7.8 ^a	2.0 ^b	5.2 ^a	7.3 ^a	4.7 ^b	80.7 ^a	49.6 ^a	3.1 ^a

In a column within each water treatment, means followed by the same superscripts are not significantly different at $P < 0.05$.

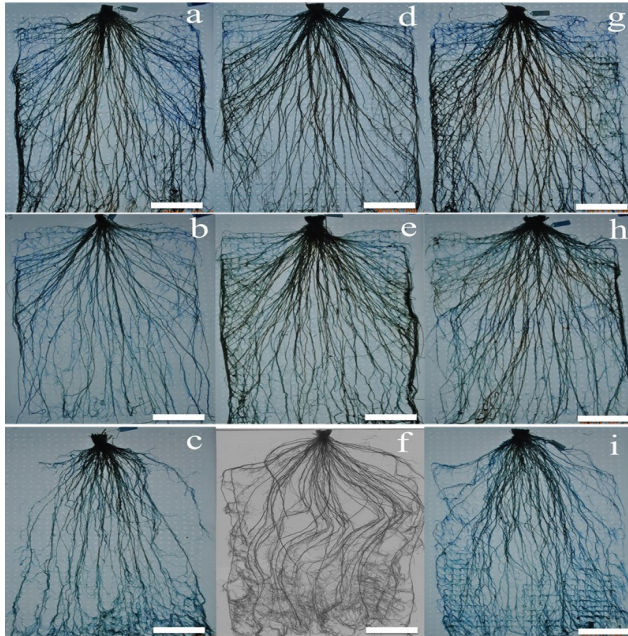


Fig. 2. Root systems of 38-d-old Nipponbare (a-c), *+qLLRN-12* (d-f), and CSSL47 (g-i) genotypes grown under well-watered, WW (a, d, g), transient drought to waterlogging, D-W (b, e, h), and transient waterlogging to drought, W-D (c, f, i) conditions. The root system was extracted using a root box pinboard method as described by Kono et al. (1987). Prior to taking of digitized images in a scanner, the root systems were stained with 0.25% of Coomassie Brilliant Blue R solution for at least 24 h. White bars = 5 cm.

genotypes was significantly higher by 47% and 22%, respectively, than that of Nipponbare under D-W (Table 1). On the other hand, only CSSL47 showed a significantly higher NR than Nipponbare by 16% under W-D. For mean length of NR, only CSSL47 showed a significantly higher value by 27% than Nipponbare under W-D. Furthermore, the total length of NR was significantly higher by 18% and 15% in *+qLLRN-12* and CSSL47, respectively, than in Nipponbare under D-W while only CSSL47 showed a significantly higher total length of NR by 15% than Nipponbare under W-D conditions.

The root system based on TRL showed a significant and positive correlation with linear frequency of LRs per unit of NRs under both D-W and W-D conditions, but not under WW control (Fig. 3). The root system based on TRL was also significantly and positively correlated with total length of LRs (0.97, $P < 0.001$) and NRs (0.88, $P < 0.01$) under D-W conditions (data not shown). Furthermore, the TRL was significantly and positively correlated with the total length of lateral roots (0.83, $P < 0.05$) but not the total length of NR under W-D conditions (data not shown).

Root Response of *+qLLRN-12* Genotype under Continuous Drought Stress

The root growth and development of *+qLLRN-12* genotype was examined after 38 d of continuous drought (C-D) condition (Table 2; Fig. 4). Relative to its WW counterparts, root system development (RSD) and shoot dry matter production of *+qLLRN-12* genotype was significantly reduced by 62.4% and 32.1% under C-D conditions (Table 2). On the other hand, the SDW and TRL of *+qLLRN-12* genotype were not significantly affected under SMF conditions (Table 2).

Nodal root production of *+qLLRN-12* genotype was generally lower in all soil moisture stress treatments relative to the WW control (Table 2). Reduction in NR production was greatest (60%) under C-D conditions. The mean length of NR *+qLLRN-12* genotype was not significantly affected by any of the soil moisture stress treatments (Table 2). On the other hand, LR development, particularly the L-type LR, was significantly greater in soil moisture stress treatments than in the WW control (Table 2). Overall, the RSD of *+qLLRN-12* genotype was more inhibited under C-D than under SMF treatments, relative to the WW control (Fig. 4).

DISCUSSION

Several studies emphasized the importance of root plasticity and its associated physiological responses under the transient cycle of soil moisture stresses such as

Table 2. Shoot dry weight and root system development of a 38-d-old *+qLLRN-12* genotype grown under well-watered (WW), transient drought-to-waterlogging (D-W) and transient waterlogging-to-drought (W-D) conditions.

Moisture Treatment	Shoot Dry Weight (g/plant)	Total Root Length (m/plant)	Lateral Roots (LR) (number LR cm ⁻¹)			Nodal Roots (NR)	
			L-type	S-type	Total	Number	Length (cm)
WW	2.09 ^a	10.2 ^{ab}	0.9 ^c	4.6 ^a	5.4 ^b	106.3 ^a	41.7 ^a
D-W	2.42 ^a	11.4 ^a	1.7 ^b	4.6 ^a	6.2 ^a	101.3 ^a	39.0 ^b
W-D	1.99 ^a	7.9 ^b	3.5 ^a	4.6 ^a	6.9 ^a	67.3 ^b	38.8 ^b
C-D	1.42 ^b	3.9 ^c	1.8 ^b	5.0 ^a	6.7 ^a	43.0 ^c	40.4 ^{ab}

In a column within each water treatment, means followed by the same superscripts are not significantly different at $P < 0.05$.

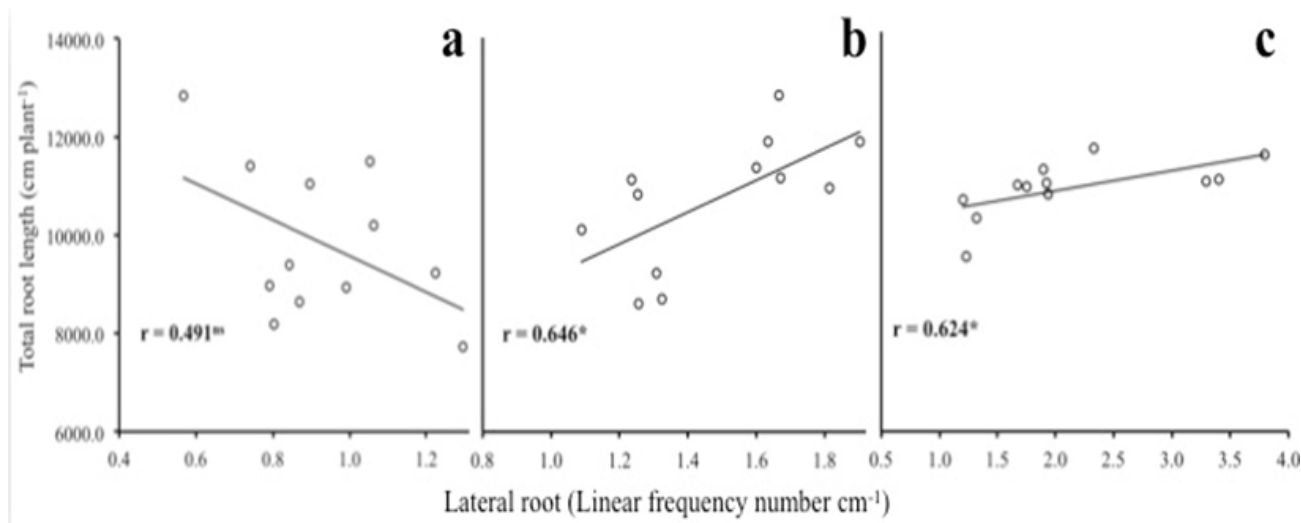


Fig. 3. Relationship between the number of lateral roots per unit length of nodal root axis and total root length under well-watered, WW (a), transient drought to waterlogging, D-W (b), and transient waterlogging to drought, W-D (c) conditions. ns, not significant and *, significant at $P < 0.05$.

waterlogging and drought (Suralta et al. 2010; Bañoc et al. 2000; Siopongco et al. 2008; Niones et al. 2012). The genetic variations in plastic root development as expressed in total root length and its root components (e.g., total nodal root length and total lateral root length) induced by SMF significantly influenced grain yield (Yamauchi et al. 1996; Bañoc et al. 2000; Suralta et al. 2008; Siopongco et al. 2008; Niones et al. 2012, 2013).

The results obtained from our series of experiments on SMF, such as in short-duration (14 d) hydroponics conditions (Suralta et al. 2008), prolonged-duration (38 d) soil-filled root box conditions (Suralta et al. 2010), and continuous cycles under field conditions (Niones et al. 2012) were consistent. The results showed that CSSL47 had greater expression of root plasticity than Nipponbare under SMF (W-D, D-W). CSSL47 was able to maintain nodal root (NR) production, promote root elongation, and L-type LR production. These conditions resulted in higher stomatal conductance and photosynthesis, and ultimately, higher shoot dry matter and yield (Suralta et al. 2010; Niones et al. 2012, 2013).

Validation of the 'Kasalath' Allele Roles in Phenotypic Plasticity in Lateral Root Development under SMF

Genotype with *+qLLRN-12* demonstrates greater L-type LR production, which led to development of larger root system under SMF (Fig. 2; Table 1). The results clearly indicate that the presence of 'Kasalath' allele was responsible for the increased production of L-type LRs, which contributed to the increase in total length of lateral root that enhanced the root system architecture during

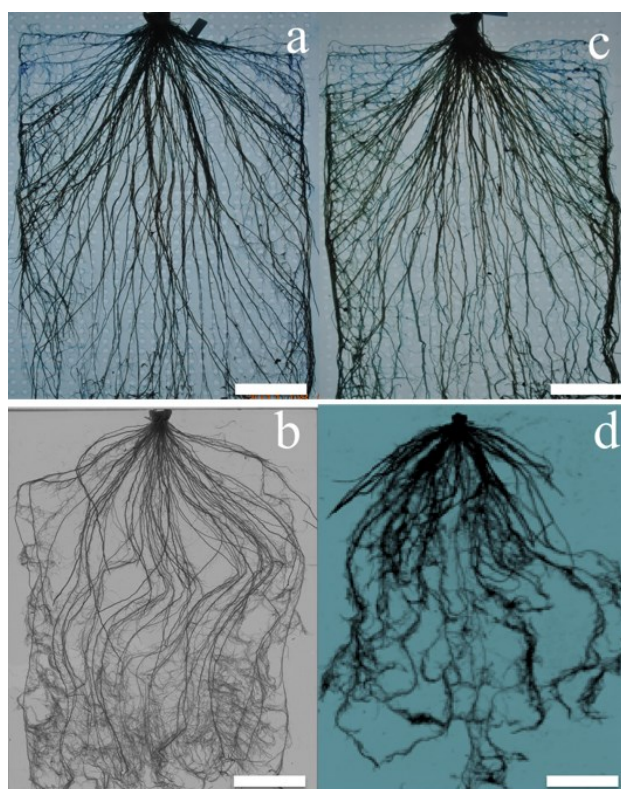


Fig. 4. Root system profile of 38-d-old *+qLLRN-12* genotype grown under a: well-watered (WWW), b: transient drought to waterlogging (D-W), c: transient waterlogging to drought (W-D), and d: continuous drought (C-D) conditions. Soil moisture content during drought (i.e., W-D, D-W, C-D) condition was maintained at 20% (v/v). The root system was extracted using a root box pinboard method as described by Kono et al. (1987). Prior to taking of digitized images in a scanner, the root systems were stained with 0.25% of Coomassie Brilliant Blue R solution for at least 24 h. White bars = 5 cm.

Table 3. Root traits and their functional characteristics that are commonly characterized in root QTL mapping studies.

Root Traits	Functional Characteristics	Reference
Root branching	Capacity to explore available nutrient and moisture in the soil	Horii et al. (2006)
Total root length	Determines the size of whole root system; major determinant for water and nutrient uptake from the soil	Qu et al. (2008) Champoux et al. (1995) Qu et al. (2008)
Root number	Influences root system architecture and physical strength	Zheng et al. (2003) Ray et al. (1996) Ali et al. (2000)
Maximum root depth	Absorption of soil moisture and nutrients in deeper soil layer	Courtois et al. (2009)
Root to shoot ratio	Determines the degree of assimilate allocation into the roots	Price et al. (2002) Zheng et al. (2003)
Root thickness	Determines penetration ability and hydraulic conductivity	Qu et al. (2008) Kamoshita et al. (2002)
Specific root length	Degree of branching of lateral roots, porosity due to aerenchyma development	Zheng et al. (2003)
Root penetration index	Ability to penetrate subsurface hardpan layer	Ray et al. (1996)
Root dry weight	Ability to permeate a large volume of soil	Horii et al. (2006)
Lateral root number	Serves to anchor the plant securely into the soil; Contributes to water and nutrient uptake	Huang et al. (2004); Zheng et al. (2003); Niones et al. (2015); Suralta et al. (2015)
L-type Lateral root number	Degree of branching, capacity of exploration; Contributes greater water and nutrient for the growth and development of the plant especially under water stress-prone environments	Niones et al. (2015); Suralta et al. (2015)

SMF at early growth stage (Fig. 1; Table 1). Enhanced whole root system contributed to increase in shoot biomass, which concurred with our earlier findings in a field experiment (Niones et al. 2015).

The Role of *+qLLRN-12* is Specific for Adaptation under SMF Stress

Our previous studies showed that shoot and root system development of CSSL47 is comparable with Nipponbare under continuous drought and waterlogged conditions (Suralta et al. 2008b; Suralta et al. 2010; Niones et al. 2012, 2013). These results indicate that CSSL47 has similar genetic control with the recurrent parent Nipponbare in terms of their root responses to drought or waterlogging or well-watered conditions. The CSSL47 and CSSL52 shared the same substituted segment from Kasalath in chromosome 12, although the latter had less chromosome segment substitution than the former CSSL. In this study, the shoot and root system development of CSSL52 was inhibited when grown continuously under drought condition for 38 d compared with the WW and SMF conditions (Table 2). Under SMF, however, CSSL52 consistently showed significantly greater root system than under continuous drought conditions especially brought about by its greater LR production particularly the L-type under both D-W and W-D conditions. This result indicates that QTL for the plasticity in LR development is unique and triggered only under SMF conditions. It was noted that the promotion of L-type LR was greater under

W-D than under D-W conditions (Tables 1 and 2) similar to our previous findings (Suralta et al. 2010; Niones et al. 2015). During periods of drought, the plasticity in L-type LR contributed to the increase in root surface area which subsequently increased soil water extractions from the drying soil (Siopongco et al. 2005, 2006; Suralta et al. 2010; Niones et al. 2015).

The significant and associated markers of *+qLLRN-12* were compared with the genes related to root development in rice from the EURoot project database (<http://euroot.cirad.fr/euroot/JSP/interface.jsp>). The suggestive and nearest possible genes related to root development, wherein the QTL (*+qLLRN-12*) in chromosome 12 region was detected, are the *OsSCR* and *PSTOL1* genes. The *OsSCR*, known as scarecrow gene, is a transcription factor that regulates an asymmetric cell division of the cortex or endodermis progenitor cell in the root, which is essential for generating the radial organization of the root (Kamiya et al. 2003). The scarecrow mutation results in roots that are missing one cell layer owing to the disruption of an asymmetric division that normally generates cortex and endodermis. On the other hand, overexpression of *PSTOL* gene acts as an enhancer of early root growth, thereby enabling plants to acquire more phosphorus and other nutrients under low-P soils (Gamuyao et al. 2012). Rice genotypes with genomic introgression containing the *PSTOL* gene show increased biomass, increased root growth, increased tiller

number and yield increases of up to 30% when they were grown in low P conditions whereas no deleterious consequences were seen when they were grown under normal soil fertility conditions (Milner et al. 2018).

Comparison of QTLs with Other Rice Mapping Populations

We further compared the QTLs we identified, with those reported in other rice mapping populations (Table 3). Some of the QTLs identified in the substituted segments of the CSSL47 genotype overlapped with the QTLs detected from other rice mapping populations such as those controlling maximum root length, root thickness below 90-cm soil depth, root penetration index, root dry weight (RDW), root branching index, and root-to-shoot ratio (RSR) (Champoux et al. 1995; Zhang et al. 2001; Price et al. 2002; Babu et al. 2003; MacMillan et al. 2006; Horii et al. 2006; Kamoshita et al. 2002a, 2002b). These QTLs are associated with RDW, RSR, and root number located on chromosome 6 (Price et al. 2002; Zheng et al. 2003, 2006), and the root number and root thickness located on chromosome 12 (Price et al. 2002). However, the reported QTLs associated with LR developments (Zheng et al. 2003; Huang et al. 2004; Horii et al. 2006; Courtois et al. 2009; Gowda et al. 2011) did not correspond with our newly identified QTLs on chromosome 12. The expression of the previously reported QTLs was detected under continuous drought or waterlogged stress. This result also suggests that the putative QTLs controlling LR development under SMF may be completely different from those that are expressed under simple waterlogging and drought stress.

CONCLUSION

The putative *qLLRN* of the 'Kasalath' allele identified on the short-arm of chromosome 12 region was confirmed to be responsible in regulating the plasticity in development of LRs. The *+qLLRN-12* genotype validated the significant roles of this QTL in response to SMF stress. The *+qLLRN-12* demonstrates greater root system development based on total root length in response to SMF. Longer TRL was attributed to greater production of L-type LR, which can effectively maintain water and nutrient uptake in soil under SMF. Moreover, the expression of *+qLLRN-12* is unique and triggered only when plants are subjected to SMF. It can therefore be said that a putative QTL may potentially be used in the marker-aided breeding program of rice varieties to enhance their adaptive ability under fluctuating soil moisture environments. Fine mapping of the target QTL is on-going to increase the precision of the marker for use in molecular-aided selection breeding for root plasticity.

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