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Quantitative Trait Loci Associated with Root Elongation Ability of Rice under Nitrogen-deficient Condition

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Nitrogen (N) is an important nutrient influencing the growth and yield in rice. Root plasticity is a key trait in the higher uptake of nutrient from the soil, especially in conditions where some major nutrients such as N is under optimal. In this study, a total of 168 BC_1F_4 mapping population from a cross between Malay 2 and US-2 and their parents were grown in hydroponics with deficient (5 µM) and sufficient (500 µM) N for 8 days to identify putative quantitative trait loci (QTLs) associated with root elongation. Eighty-three (83) markers were used in QTL detection using composite interval mapping. Results showed that US-2 had significantly greater shoot length and number of nodal roots than Malay 2 under N-deficient conditions. Around 19.6 and 26.8% of the population in N-deficient and N-sufficient conditions, respectively, had either longer or similar seminal root length compared with US-2. For the allele distribution, 43.1% of the population had homozygous allele for Malay 2, 20.9% had homozygous allele for US-2, and 8.4% had heterozygous allele for both parents. A total of eight putative QTLs associated with seminal root length (*qSRL11.1* and *qSRL2.1*); number of nodal roots (*qNNR6.1, qNNR11.1*, and *qNNR11.2*); root dry weight (*qRDW11.1* and *qRDW11.2*); and shoot length (*qSL2.1*) in chromosome 2, 6, and 11 regions were detected under both N treatments.

Keywords: homozygous allele, hydroponics, N-deficient, QTL, root plasticity, seminal root

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most globally important cultivated crops and staple food for more than half of the world's population. However, it is also one of the most vulnerable cultivated crops to climate change (GRiSP 2013). Philippine rice production areas have expanded to about 15.8% (4.4 million hectares) in 2010 (GRiSP 2013). This production area, however, is not enough to support the increasing rice demand – therefore leading to importation of rice from neighboring rice-producing countries, particularly Thailand and Vietnam (Tibao 2009). Clearly, improving the adaptation of crops to

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climate change is one of the important strategies to support the domestic demand for rice with minimal importation.

N is one of the essential macronutrients for crop growth and development. Ammonium (NH_4^+) rather than nitrate (NO_3^-) is the major form of N present in irrigated paddy fields (Yamaya and Oaks 2004, Obara *et al.* 2010). N is important in leaf development and sink size in terms of panicle number, spikelet number, and number of filled spikelets, which largely determine the yield potential in rice.

Root system architecture (RSA) is important for supporting the above-ground development of plants through efficient uptake of water and nutrients. Improved RSA contributes to high efficiency in the acquisition of water and nutrients to support high yield production in both the unfavorable (de Dorlodot *et al.* 2007) and favorable plant growing conditions (Gewin 2010). The RSA in terms of root length, weight, number, thickness, and density of primary, lateral, and adventitious root traits is highly influenced by environmental factors such as soil moisture conditions (Suralta *et al.* 2018) and nutrient availability (Obara *et al.* 2011). Further understanding on the genetic control of root system development in response to different environmental conditions (Lynch 1995, Obara *et al.* 2011) can support the efficient crop breeding program such as those in rice (Suralta *et al.* 2018).

Root elongation is generally inhibited with an increase in exogenous NH_4^+ concentration. Studies on the genetic adaptation of rice (Obara *et al.* 2011, Ogawa *et al.* 2014, Zhao *et al.* 2014, Kim *et al.* 2015) and maize (Liu *et al.* 2007, Bonifas and Lindquist 2009, Pestsova *et al.* 2015) to soil N status, including the identification of underlying QTLs for root elongation responses to such condition have been done. These studies showed that genetic variation in the root length among four rice varieties and greatly varied depending on NH_4^+ concentration, suggesting that changes in exogenous NH_4^+ concentrations influenced rates of root elongation (Tanaka *et al.* 1993, Obara *et al.* 2011).

Studies on QTL analysis associated with root traits have gained increased attention in rice and maize. Several QTL studies associated with root elongation using diverse rice varieties were done under a wide range of NH_4^+ in hydroponics conditions (Feng *et al.* 2010; Obara *et al.* 2010, 2011, 2014; Kim *et al.* 2015).

The use of recombinant inbred line (BC_1F_4) mapping population derived from US-2 x Malay 2 crosses has been useful in detecting QTLs for blast resistance (Gandalera 2017). Malay 2 is an improved upland advanced breeding line that has a broad spectrum of blast resistance and wide adaptation and tolerance to water-scarce, upland growing conditions (Niones *et al.* 2007). On the other hand, US-2 is a universal susceptibility for blast disease but has a unique rooting ability in terms of elongation and production of nodal roots under N-deficient growing conditions. Thus, utilization of existing RIL in this study would provide a new source of genes, particularly for root elongation ability that could be used in rice breeding program targeting an improved N use efficiency (NUE). Thus, this study aimed to identify putative QTL associated with root elongation and nodal root production in response to N-deficient growing condition during the seedling stage.

MATERIALS AND METHODS

Plant Materials

A total of 168 BC_1F_4 recombinant inbred lines (RIL) derived from US-2 and Malay 2 crosses were used in this study. Malay 2 is an improved *indica* upland rice variety developed by PhilRice Midsayap Experimental Station in North Cotabato, Philippines. This promising variety has high resistance to leaf blast and moderate tolerance to drought (Niones *et al.* 2007, Gandalera 2007). On the other hand, US-2 is a universal blast susceptible rice genotype derived from *indica* and *japonica*-type crosses (Kobayashi *et al.* 2007).

Pre-germination of Seeds

Seed preparation and pre-germination were done following the method of Obara *et al.* (2010) with some modifications. The seeds of each parent, US-2 and Malay 2, and each of the 168 BC_1F_4 lines was properly labeled and placed in a small net bag. The seeds were soaked in water at 60 °C for 10 min with gentle shaking and then washed for 10 min with running tap water to cool down the seeds. The seeds were pre-germinated in a Petri dish lined with moistened filter paper and then incubated at 30 °C for 35–40 h.

Preparation of Hydroponics Solution

The hydroponics solution was prepared following the method of Obara *et al.* (2010). To maintain the pH of the nutrient solution, 400 ml of 2-(N-Morpholino) ethanesulfonic acid (MES) was added to each container with 40 L of tap water and stirred gently. Then, 10 ml of each stock solution of nutrients ($NaH_2PO_4{\cdot}2H_2O$, 0.6 M; K_2SO_4, 0.3 M; $CaCl_2{\cdot}2H_2O$, 0.3 M; $MgCl_2{\cdot}6H_2O$, 0.6 M; EDTA-Fe, 0.045 M) and 10 ml of minor reagents consisting of H_3BO_3, 0.05 M; $MnSO_4{\cdot}5H_2O$, 0.009 M; $CuSO_4{\cdot}5H_2O$, 0.0003 M; $ZnSO_4{\cdot}7H_2O$, 0.0007 M; $Na_2MoO_4{\cdot}2H_2O$, 0.0001 M was added in the container and then gently stirred. For the N-deficient concentration

(5 µM), 200 µl 1 M: NH_4Cl and for the N-sufficient (500 µM), 20 ml 1 M: NH4Cl was added in each container then gently stirred. Finally, the pH of the solution was adjusted to 5.45–5.55 by adding 1 M HCl.

Seed Sowing and N Treatment Imposition

Three pre-germinated seeds from each of the parents and BC_1F_4 lines were sown on a nylon mesh net with polystyrene support. The parents were placed at the side-edge, corner, or center across four nylon mesh nets. Each nylon mess net has six rows and consisted of 24 seeds per row. Four nylon mess nets were placed in each container on a two-by-two configuration. The containers were then covered with plastic sheets with small holes to avoid direct sunlight. Plastic sheets were removed in the afternoon, a day after sowing. Hydroponics solutions were replaced during the 4th and 6th days after sowing to maintain the pH level within 5.45–5.55. Seedling of each parent and BC_1F_4 line were grown for eight days in a 40 L hydroponics solution under either N-sufficient or N-deficient conditions. The experiment was repeated thrice which served as replications. The conditions inside the screenhouse during the conduct of each experimental replication had average temperatures of 27.3, 26.4, and 26.6 °C, respectively, while the relative humidity was 82.2, 78.0, and 79.6%, respectively.

Phenotypic Evaluation

Eight days after sowing, the root length was measured manually from the base of the shoot to the tip of the seminal root using a ruler. The length of the shoot was measured manually from the base to the tip of the longest leaf using a ruler. The number of nodal roots at the base of each seedling was counted manually. Thereafter, the whole root system was collected, placed in an envelope and then oven-dried at 70 °C for three days and weighed using an analytical balance for root dry weight.

Genotyping and QTL Identification

All of 168 BC_1F_4 mapping populations derived from US-2 and Malay 2 crosses were genotyped. Specifically, DNA extraction was done using the PhilRice Genomic DNA Extraction Protocol (Perez *et al.* 2012). The collected leaf samples from each line were placed in properly-labeled 2 ml microtubes and then pulverized using liquid N with the aid of blue micropipette tips. A total of 750 µl of pre-warmed 2x cetyl trimethylammonium bromide and 50 µl 20% SDS were added to the ground leaf samples – mixed thoroughly by vortexing and then incubated in a water bath at 60 °C for 30 min to 1 h. Microtubes were cooled briefly and then 750 µl of chloroform was added. The samples were thoroughly mixed in a vortex and then centrifuged at 10,000 rpm for 30 min. The aqueous phase was transferred into a new 1.5 ml tube and then 600 µl of ice-cold isopropanol was added. The microtubes were incubated at –20 °C overnight. After incubation, microtubes were centrifuged at 10,000 rpm for 10 min. The isopropanol was removed and then 500 µl of 70% ethanol was used to wash the DNA pellet. The microtubes were centrifuged again at 10,000 rpm for 3 min and then the alcohol was discarded. The microtubes were inverted in a paper towel to get rid of the excess liquid alcohol. The pellets were dissolved in 100 µl of TE buffer with RNase. The tubes were incubated at room temperature for 2–3 h or until pellet dissolved completely. The quality of the DNA was checked using 1.5% agarose gel electrophoresis.

Out of 138 SSR markers surveyed, 101 were identified as polymorphic markers. Eighty-three (83) polymorphic markers were distributed across 12 chromosomes and used to generate a linkage map (Figure 3) using Qgene software v.4.3.10 (Nelson 1997). QTL mapping analysis

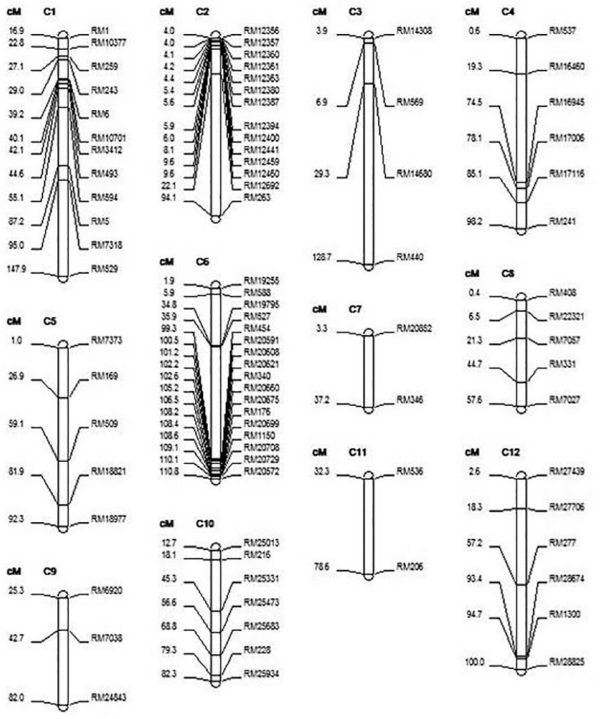

Figure 3. Linkage map of 83 polymorphic SSR markers across chromosomes in rice generated using WinQTLCart 2.5. The left side of the chromosome indicates the SSR marker loci while the right side of the chromosome indicates the name of SSR marker.

was done using Qgene software v.4.3.10 (Nelson 1997) and WinQTLCart 2.5 (Wang *et al.* 2012). The putative QTLs were detected using composite interval mapping with the critical threshold value of the logarithm of odds (LOD) score set at 2.5 to detect the presence of associated QT (Lander and Botstein 1989, Nelson 1997).

RESULTS AND DISCUSSION

Phenotypic Evaluation of Parents and BC_1F_4 Mapping Population

Relative to the N-sufficient control, US-2 had a decrease in shoot length by 1.4% but had an increase in seminal root length (45.8%), number of nodal roots (5.4%), and root dry weight (50%) under N-deficient condition. On the other hand, Malay 2 had an increase in shoot length by 23.1% as well as seminal root length (37.5%), number of nodal roots (10.2%), and root dry weight (38.3%) under N-deficient condition. US-2 had significantly greater shoot length than Malay 2 under N-deficient condition, but the former had a greater number of nodal roots than the latter genotype under both N treatments (Figure 1; Table 1). In Malay 2, there were no significant differences between N treatments in all traits measured (Table 1). US-2 had higher ability to elongate its roots than Malay 2 under N-deficient condition, which indicates wide genetic variations between these two parents under N-deficient condition. The present results showed similar trends to the earlier findings of Obara *et al.* (2010), wherein gradual reductions in root length in both Koshihikari and Kasalath were observed when NH_4^+ concentration of the growing medium was increased. Kim *et al.* (2015) also reported variations in root length and root weight of Ilpumbyeo and Moroberekan under hydroponic condition with exogenous NH_4^+ – an indication of genetic variation of these traits between the two genotypes. Under N-deficiency, carbohydrates were accumulated in shoots, whereas root systems expanded to increase their potential for nutrient acquisition as their adaptive response to nutrient stress (Cai *et al.* 2011). Removal of N for three weeks and P, K, and Mg for four weeks from the culture solution significantly decreased the dry matter production and changed root morphology of rice relative to its counterparts with adequate nutrient supply (Cai *et al.* 2011). Furthermore, under N and P deficiency, shoot growth was significantly decreased and thus increased root to shoot biomass ratio; under K and Mg deficiency, shoot growth was not affected and thus, root to shoot biomass ratio was decreased (Cai *et al.* 2011).

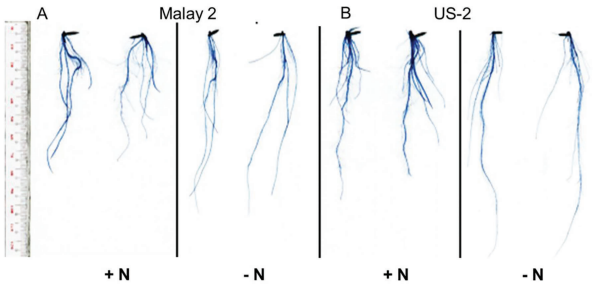

Figure 1. Root system of Malay 2 (A) and US-2 under (B), + N: sufficient (500 µM NH_4^+) and – N: deficient (5 µM NH_4^+) conditions at eight days after sowing.

Frequency Distribution of BC_1F_4 Mapping Population

Under N-deficient condition, the seminal root length among 168 BC_1F_4 lines ranged 34–167 mm with a mean value of 89 mm. The number of nodal roots, root dry weight, and shoot length of the population ranged 4–7 mm, 0.96–6.7 mg, and 68–222 mm, respectively. Under N-sufficient condition, on the other hand, seminal root length of the population ranged 36–119 mm while mean values of the population for number of nodal roots, root dry weight, and shoot length were 5 mm, 1.7 mg, and 178 mm, respectively (Table 1). The mapping population showed wide and normal frequency distribution of seminal root length, number of nodal roots, root dry weight, and shoot length (Figure 2). Most number of individuals lies in the mean value range of the parent lines that are suitable for QTL mapping (Zhao *et al.* 2014) of the traits.

Table 1. Root trait responses of two parents and frequency distribution of 168 BC_1F_4 recombinant inbred line population grown in hydroponics conditions to two different N concentrations.

Trait	N Treatment	US-2	Malay 2	BC_1F_4 RIL Population		
				Min	Max	Mean
Seminal root length (mm)	+ N	79.3[b]	64.0[a]	36	119	69
	– N	115.7[a] (+45.9%)	88.0[a] (+37.5%)	34	167	89
Nodal roots (no. seedling^{-1})	+ N	6.3[a]	3.3[a]*	4	8	5
	– N	6.7[a] (+6.3%)	3.7[a]* (+12.1%)	4	7	5
Root dry weight (mg)	+ N	1.9[a]	1.2[a]	0.78	4.3	1.7
	– N	2.8[a] (+47.4%)	1.7[a] (+41.7%)	0.96	6.7	2.4
Shoot length (mm)	+ N	186.0[a]	108.3[a]	84	231	178
	– N	183.3[a] (–1.5%)	83.3[a]* (–23.1%)	68	222	146

+ N: sufficient (500 µM NH_4^+); – N: deficient (5 µM NH_4^+)
Means followed by the same letters are not significantly different among two NH_4^+ concentration for each trait (*t*-test, $P < 0.05$)
*Significant difference in traits between two parents (*t*-test, $P < 0.05$).

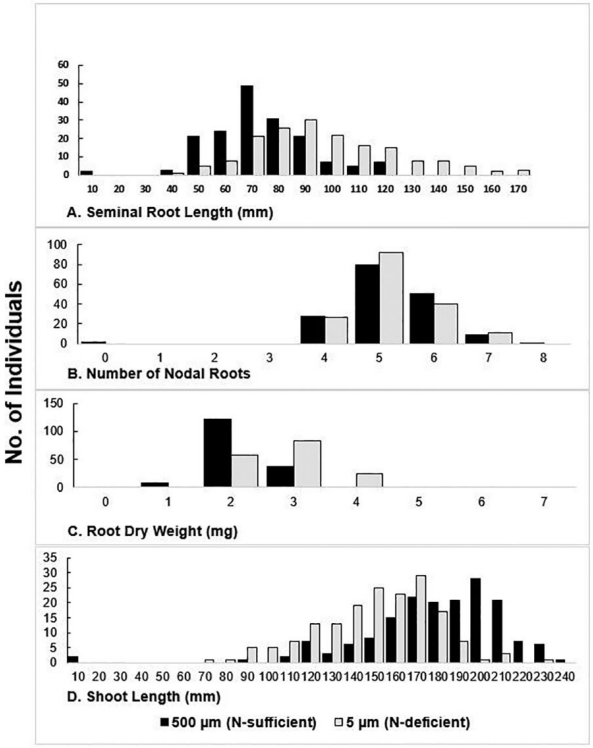

Figure 2. Frequency distribution of (A) seminal root length, (B) number of nodal roots, (C) root dry weight, and (D) shoot length in 168 Malay-2/US-2 BC_1F_4 lines grown in hydroponics with either N-sufficient (500 µM NH_4^+) or N-deficient (5 µM NH_4^+) conditions.

Detection of QTL Associated with Seminal Root Elongation

Eight putative QTLs were detected in three chromosomes with an LOD score of 2.5 under – N (deficient) and + N (sufficient) conditions.

Under N-deficient condition, QTLs for seminal root length (*qSRL11.1*), number of nodal roots (*qNNR11.1*), and root dry weight (*qRDW11.1*) in chr 11 region while one QTL for number of nodal roots (*qNNR6.1*) in chr 6 region were detected (Table 2). The QTLs in chr 11 region were located near the flanking markers of RM536 and RM206 locus with a distance of 46.31 cM and LOD value of 3.45, 3.51, and 5.99, respectively (Figure 4B and C). On the other hand, the QTL (*qNNR6.1*) detected in chr 6 was located near the flanking markers of RM340 and RM20660 (72.0 cM) locus with LOD value of 2.50 (Figure 4B). The phenotypic variance of *qSRL11.1*, *qNNR11.1*, and *qRDW11.1* were 0.09, 0.09, and 0.15, respectively – while that of *qNNR6.1* was 0.07. The estimated position of *qSRL11.1*, *qNNR11.1*, and *qRDW11.1* were 70.3 cM, 78.3 cM, and 76.3 cM, respectively, with flanking markers between RM536 and RM206 locus – while *qNNR6.1* was located at 103.9 cM between RM340 and RM20660 locus. The additive effects of four QTLs detected were all negative, indicating that the alleles came from the US-2 parent.

Under N-sufficient condition, one putative QTL each for seminal root length (*qSRL2.1*) and shoot length (*qSL2.1*) in chr 2 region, and number of nodal roots (*qNNR11.2*) and root dry weight (*qRDW11.2*) in chr 11 region were detected (Table 2; Figure 4A and C).

The two QTLs in chr 2 region were located at 93.9 cM between RM12692 and RM263 locus, while the QTL in chr 11 region was located at 78.3 cM between RM536 and RM206 locus (Figure 4A and C). The contributing allele for *qSRL2.1* and *qSL2.1* was from Malay 2 with additive effects of 7.37 and 12.33, respectively. The QTLs for number of nodal roots located in chr 11 region can be categorized as constitutive QTL since it was detected under both N conditions.

According to Obara *et al.* (2010), a supplement of NH_4^+ alone is adequate for mapping constitutive QTL

Table 2. QTLs associated with different root traits detected in US-2/Malay 2 BC_1F_4 mapping population.

Traits	QTL	N treatment	Chr	Flanking markers		QTL position (cM)	LOD	Additive effect	Allele	R^2
				Left marker	Right marker					
Seminal root length	*qSRL2.1*	+N	2	RM12692	RM263	93.9	***4.03***	7.37	Malay 2	0.10
	qSRL11.1	–N	11	RM536	RM206	70.3	***3.45***	–26.38	US-2	0.09
Number of nodal roots	*qNNR11.1*	-N	11	RM536	RM206	78.3	***4.60***	–0.62	US-2	0.12
	qNNR11.2	+N	11	RM536	RM206	78.3	***3.51***	–0.43	US-2	0.09
	qNNR6.1	–N	6	RM340	RM20660	103.9	***2.50***	–0.29	US-2	0.07
Root dry weight	*qRDW11.1*	–N	11	RM536	RM206	78.3	***5.94***	0.00		0.15
	qRDW11.2	+N	11	RM536	RM206	76.3	***5.99***	–0.001	US-2	0.15
Shoot length	*qSL2.1*	+N	2	RM12692	RM263	93.9	***3.84***	12.33	Malay 2	0.10

+ N: sufficient (500 µM NH_4^+); – N: deficient (5 µM NH_4^+)
SRL: seminal root length (mm); NNR: number of nodal roots per seedling; RDW: root dry weight (mg plant-1); and SL: shoot length (mm)

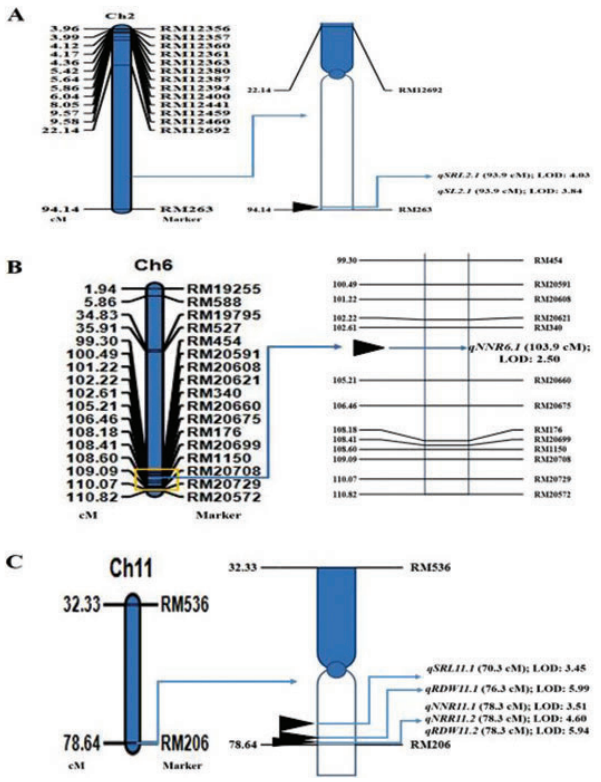

Figure 4. Locations of QTLs at (A) chromosome 2 (*qRL2.1* and *qSL.2.1*); (B) chromosome 6 (*qNNR6.1*); and (C) chromosome 11 (*qRL11.1*, *qRDW11.1*, *qNNR11.1*, *qNNR11.2*, and *qRDW11.2.*) using US-2/Malay 2 BC_1F_4 populations. Black arrowheads indicate the approximate QTL location using ICIMapping 4.0.

controlling seminal root length in response to NH_4^+ concentration. Generally, QTL are categorized into two types: constitutive QTL (which is active under favorable condition) and adaptive QTL (which respond to changes in environmental conditions) (Collins *et al.* 2008). Previous studies showed that most of the QTL associated with root traits of rice in response to changes in N concentrations were detected across 12 chromosomes while in maize, QTLs were detected in chr 1, 2, 3, 4, 5, 7, 8, and 9 regions (Feng *et al.* 2010; Obara *et al.* 2010, 2015; Kim *et al.* 2015). Seven QTLs associated with N-deficiency tolerance were detected in chr 1, 2, 3, and 8 regions using R9308/ Xieqingzao B RIL population (Feng *et al.* 2010). The N-deficiency tolerance was assessed using shoot dry weight, root dry weight, total plant dry weight, maximum root length, chlorophyll content, and plant height. Kim *et al.* (2015) detected seven QTLs for root dry weight under 0, 250, and 500 NH_4^+ concentrations, and root length under 0 and 500 NH_4^+ concentrations in chr 1, 2, 4, 6, 8, and 10 regions. Obara *et al.* (2010) detected and identified *qRL6.1* under 5 µM NH_4^+ using Koshihikari and Kasalath chromosome segment substitution lines. In addition, five putative QTLs (*qRL5.1-YP1* and *qRL5.2-YP10* in chr 5, *qRL6.3-YP10* and *qRL6.4-YP5* in chr 6, and *qRL7.1-YP1* in chr 7) affecting root elongation in seedlings grown under hydroponic condition without N supply were identified using IR64 introgression lines (Obara *et al.* 2014). However, no QTL for number of nodal roots located at has been identified from the above studies, indicating that these QTLs might be new and should be further validated in future studies.

Allele Distribution of BC_1F_4 Population

In QTL in chr 2 regions, 29.2% of the population had homozygous allele for Malay 2, 6.0% carried homozygous allele for US-2, and 17.9% had heterozygous allele of Malay 2 and US-2. In chr 6, 10.7% carried homozygous allele for Malay 2, 79.8% homozygous for US-2, and 4.8% heterozygous for both parents. Furthermore, in chr 11, a total of 89.3% of the population carried homozygous allele for Malay 2, 1.2% homozygous for US-2, and 2.4% heterozygous for both alleles.

We detected eight QTLs associated with root development on three chromosome regions and their corresponding effects contributed by either of the parents (Figure 5). The BC_1F_4 lines carrying QTL in chromosome 2, 6, and 11 regions with effects either from US-2 or Malay 2 allele

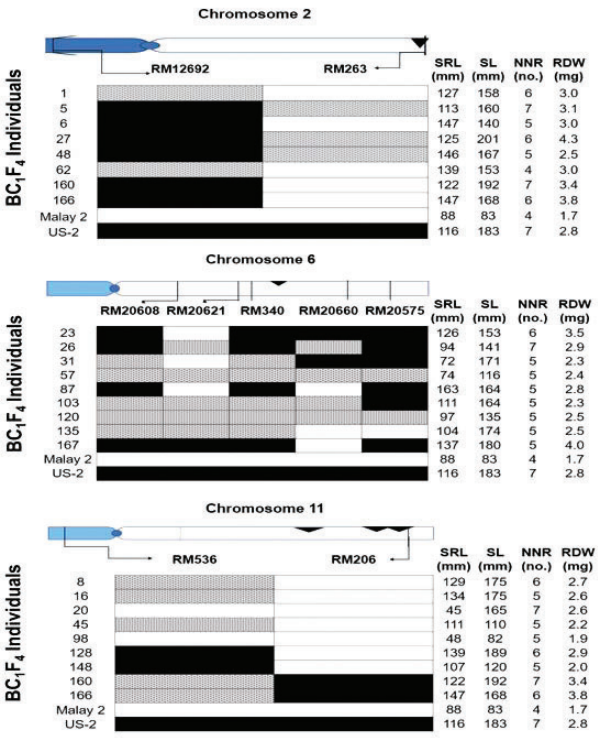

Figure 5. Allele distribution of BC_1F_4 of putative QTL regions showing homozygous Malay 2 allele (□), homozygous US-2 allele (■), and heterozygous allele (▒) between the two parents. Black arrowheads (▼) indicate the approximate QTL location.

were classified and selected based on their genotypic data (Figure 5). The homozygous US-2 allele was observed in six lines at chr 2, six lines at chr 6, and four lines at chr 11 regions. On the other hand, homozygous Malay 2 allele was observed in five lines at chr 2, five lines at chr 6, and seven lines at chr 11 regions.

Specifically, lines 23, 87, and 167 in RM340 loci at chr 6 and lines 160 and 166 in RM206 loci at chr 11 with introgressed US-2 allele had comparable lengths in seminal root and shoot, and number of nodal roots with US-2 (Figure 5). Although line 26 had introgressed segment of US-2 allele at chr 6, it had a comparable number of nodal roots but shorter shoot and root lengths than US-2. The results implied that the presence of US-2 allele may significantly contribute to either the elongation of shoots and seminal root as or production of nodal roots in response to different N conditions.

Furthermore, lines 1, 6, 62, 160, and 166 in RM263 loci at chr 2 and lines 8, 16, 45, 128, and 148 in RM206 loci at chr 11 had homozygous allele of Malay 2 and had comparable root and shoot length with US-2 (Figure 5). On the other hand, we also noted other lines such as line 106 in RM263 loci at chr 2 and line 156 in RM206 loci at chr 11, which had the homozygous segment of Malay 2 allele showing shorter shoot and seminal root lengths and less number of nodal roots than US-2 (data not shown). The above results also demonstrated that not all alleles can be expressed in individual phenotype due to the effect of external factors (*i.e.*, environment) that influence the expression of genes or traits. The continuous variations for complex traits such as root elongation ability were due to genetic complexity that arose from segregating alleles at multiple loci and environmental sensitivity (Mackay 2009). The effect of each of these alleles on phenotype is often relatively small, and their expression is sensitive to the environment. Because of this complexity, many genotypes can give rise to the same phenotype within the same environments or different phenotypes among different environments (Mackay 2009).

Comparison of Detected QTLs to other Mapping Populations

Putative QTLs identified from US-2 and Malay 2 crosses were compared to the reported QTLs detected from other mapping populations (Table 3). Five QTLs associated with seminal root length under N-sufficient condition were detected in chr 1, 6, 8, 11, and 12 regions, while

Table 3. Comparison of identified QTL associated with root elongation from other mapping populations.

Trait	Chr	Marker	Loci (Mbp)	Population	Reference
Seminal root length under 500 μM NH_4+ condition	1	R210–R2417	10.54–33.18	Koshihikari/Kasalath	Obara *et al.* 2010
	6	R2549–R1167	25.79–32.12	Koshihikari/Kasalath	Obara *et al.* 2010
	8	S2104–C390	0–1.59	Koshihikari/Kasalath	Obara *et al.* 2010
	11	G320–G1425	6.67–26.51	Koshihikari/Kasalath	Obara *et al.* 2010
	12	S10637A–R1709	2.19–23.70	Koshihikari/Kasalath	Obara *et al.* 2010
Seminal root length under 5 μM NH_4^+ condition	1	C1370–C112	31.21–44.75	Koshihikari/Kasalath	Obara *et al.* 2010
	2	C132–C499	5.80–21.74	Koshihikari/Kasalath	Obara *et al.* 2010
	4	R2373–C445	0–36.06	Koshihikari/Kasalath	Obara *et al.* 2010
	6	R2549–R1167	25.79–32.12	Koshihikari/Kasalath	Obara *et al.* 2010
Root elongation without N supply	5	RM163	19.3	IR64/IR65600-87-2-2-3	Obara *et al.* 2014
	5	RM163	19.3	IR64/IR71195-AC1	Obara *et al.* 2014
	6	RM3827	22.3	IR64/IR71195-AC1	Obara *et al.* 2014
	6	RM3138	28.5	IR64/IR69093-41-2-3-2	Obara *et al.* 2014
	7	RM248	29.3	IR64/IR65600-87-2-2-3	Obara *et al.* 2014
Relative maximum root length	1	RM5385–RM7192	–	R9308/Xieqing-zao B	Feng *et al.* 2010
Maximum root length under high N supply	1	RM104–RM14	–	93-11/Nipponbare	Zhao *et al.* 2014
	4	RM317–RM5473	–	93-11/Nipponbare	Zhao *et al.* 2014
	3	RM6080–RM411	–	93-11/Nipponbare	Zhao *et al.* 2014
Maximum root length under low N supply	4	RM6748–RM5473	–	93-11/Nipponbare	Zhao *et al.* 2014
Root dry weight under low N supply	9	RM1189–RM257	–	93-11/Nipponbare	Zhao *et al.* 2014

four QTLs associated with seminal root length under N-deficient conditions were detected in chr 1, 2, 4, and 6 regions. Furthermore, seven QTLs associated with root elongation without N supply were detected in chr 5, 6, and 7 regions. Other QTLs associated with relative maximum root length, maximum root length, and root dry weight under both high and low N supply were detected in chr 1, 3, 4, and 9 regions.

The putative QTL *qRL11.1* detected in US-2 and Malay 2 crosses lie within the loci range of QTL associated with seminal root length in 500 µM N at 6.67–26.51 Mbp (Obara *et al.* 2010) but detected under N-deficient conditions only. The putative QTLs found in US-2/Malay 2 mapping population in chr 2, 6, and 11 regions were completely different from those found in other mapping populations.

CONCLUSION AND RECOMMENDATIONS

The modified hydroponic basal nutrients solution with different N concentrations technique of Obara *et al.* (2010) can be used for reliable and rapid phenotyping of root elongation ability in rice. Wide variations in root and shoot responses between US-2 and Malay 2 and among their mapping populations were observed using two NH_4^+ concentrations. Although US-2/Malay 2 RIL mapping population was developed and intended for blast studies, the US-2 can be a promising donor for improving root elongation ability and adaptation under limited N growing environments. Four putative QTLs (*qRL11.1, qNNR11.1, qRDW11.1*, and *qRNN6.1*) associated with root development under N-deficient conditions were detected in chr 11 and 6 regions. Furthermore, four QTLs (*qSRL2.1* and *qSL2.1*) in chr *2* and two (*qNR11.2, qRDW11.2*) in chr 11 regions associated with root development under N- sufficient condition were also detected. These QTLs can be used for further validation and/or development of marker systems useful in breeding programs aiming for improved adaptation of rice to growing environments with limited N.

To better able to visualize and quantify the RSA for better understanding of the response of US-2 and Malay 2 and its mapping population under N-deficient condition, root phenotyping until vegetative stage using rootbox pinboard method will be done. Saturation with SSR markers and fine mapping wherein putative QTLs detected is subject for future research to generate robust and tightly linked markers useful in marker-aided selection breeding program for NUE.

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