Comparative mapping of rice root traits in seedlings grown in nutrient or non-nutrient solution*

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Abstract   The component and amount of nutrient in the growth medium are the major factors affecting root growth. For the systematic dissection of root gene expression, evaluation of nutrient and non-nutrient solutions was conducted for their effect on root traits and quantitative trait loci (QTL) mapping. Three rice root parameters, maximum root length (MRL), root dry weight (RDW), and root/shoot ratio of dry weight (RSR), were characterized within a double haploid (DH) population from a cross of ZYQ8 (*indica*) and JX17 (*japonica*). The value of the three root traits in two parents all decreased under the nutrient condition compared to those under the non-nutrient condition, of which RSR decreased up to 2.6-fold on average. In the DH population, more than 70% lines in MRL, 94% lines in RDW, and all the lines in RSR were scored lower. In total, eight QTLs were identified in nutrient system (5 from JX17 alleles and 3 from ZYQ8 alleles) while five QTLs were detected in non-nutrient system (4 from JX17 alleles and 1 from ZYQ8 alleles). Of them, one QTL for RSR was shared by both culturing systems, seven QTLs were specific in nutrient system and the other four QTLs were specific in non-nutrient system. All 13 QTLs were distributed over 7 rice chromosomes—2, 3, 4, 5, 6, 9 and 10, respectively.

Keywords: mapping, nutrient, quantitative trait loci (QTL), rice, root traits.

Root growth, which widely influences the plant productivity, ability of drought resistance, grain yield, and other important agronomic traits, could be strongly affected by its genetic characteristics, physical environments and crop management practices[1]. An advanced root system functions in water conservation and water collection that are regulated by a number of major and minor genes in rice[2]. Mapping quantitative trait loci (QTLs) of rice root traits has been conducted in several laboratories with different mapping populations. In 1995, Champoux analyzed five parameters of rice root morphology[3] such as maximum root length, root weight, deep root weight, root/shoot ratio and root thickness for a recombinant inbred (RI) population of CO39 (rainfed lowland)/ Moroberekan (upland) grown in soil. In three replicated field experiments with the differences in planting time, temperature and water supply, a total of 203 RI lines were planted and their root traits were evaluated at seedling, early vegetative, and late vegetative growth stage. Mapping results revealed that QTLs identified in different environments or growing stages were much varied. Similar results were also displayed in Price's[4] and Zheng's[5] reports.

Among the environmental factors influencing QTL expression or plant physiological reaction, nutrient is the most important one that could seriously affect rice root parameters[6]. In *Arabidopsis*, Malamy and Ryan[7] demonstrated that both the number and placement of lateral roots are highly responsive to nutritional cues. For the evaluation of the effect of nutrient on root production in rice, the study here conducted two culturing systems, nutrient and non-nutrient culturing solutions, within a double haploid (DH) population from a cross of ZYQ8 (*indica*) and JX17 (*japonica*). Phenotypic data for each line was scored in seedlings and QTLs were identified and compared between these two culturing systems.

1 Materials and methods

1.1 Rice materials

ZYQ8 (*indica*) and JX17 (*japonica*) were two Chinese rice cultivars. A permanent DH population containing 127 lines had been developed from a cross of ZYQ8 and JX17, and its molecule linkage map with 233 molecular markers had been constructed and widely used in the qualitative and quantitative trait gene mappings[8~10].

1.2 Root trait investigation

Rice seeds were surface sterilized in 3% of H_2O_2

for 10 min, then washed with distilled water three times. After soaking into water at 37 ℃ for two days, six germinated seeds for each individual line were planted in a pot of 3 cm diameter on a floating foamed plastic. All DH lines were placed into a polyethylene vessel fully filled with distilled water or nutrient solution. One liter of nutrient solution includes 114 mg NH_4NO_3, 89 mg K_2SO_4, 406 mg $MgSO_4 \cdot 7H_2O$, 147mg $CaCl_2 \cdot 2H_2O$, 50 mg $NaH_2PO_4 \cdot 2H_2O$, 1.8011 mg $MnCl_4 \cdot H_2O$, 0.0920 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.0442 mg $ZnSO_4 \cdot 7H_2O$, 1.124 mg H_3BO_3, 0.0390 mg $CuSO_4 \cdot 5H_2O$, 27.85 mg $FeSO_4 \cdot 7H_2O$, and 37.25 mg Na_2EDTA[11]. Both growing solutions were changed every other day. The culture room was kept at 25~28 ℃ with 17 h fluorescent lighting per day. After ten days' growth, rice seedlings were carefully pulled out. Its maximum root length (MRL) was measured for the mean value of each line. The shoot and roots for each line were placed in envelopes and dried at 65 ℃ for two days. Dry weights were used to compute root dry weight (RDW) and root/shoot ratio of dry weight (RSR), respectively.

1.3 QTL detection

The data of MRL, RDW and RSR for each line were individually input into the mapping database of the DH population. A LOD score of 2.5 was used as the threshold value for the possible QTLs determination with MAPMAKER/QTL Version 1.1b[12].

2 Results

2.1 Root trait evaluation in two culturing systems

Phenotypic data for two parents and DH lines in each trait/culturing solution combination are summarized in Table 1. The *indica* parent, ZYQ8, had a little advanced root system than the *japonica* parent, JX17. The values of MRL and RDW in two parents are both larger than the corresponding mean value of all the DH lines, whereas the values of RSR in two parents are similar to the mean in the DH population. All the three traits in two culturing systems are distributed continuously, and the range of the trait value in the DH lines is very wide, which could be well suitable for possible QTL detection. The frequency distributions of the three traits in the two culturing systems among DH lines are shown in Fig.1.

Table 1. The measurements of the three root traits in rice in nutrient (NS) or non-nutrient solution (NNS)

Traits	MRL (cm)		RDW (mg)		RSR	
	NS	NNS	NS	NNS	NS	NNS
Parents:						
ZYQ8	12.58	16.00	2.87	3.70	0.22	0.58
JX17	11.92	13.33	3.05	3.42	0.21	0.60
DH lines:						
Range	4.63~14.00	6.25~16.50	1.03~3.41	1.48~4.80	0.10~0.33	0.40~0.85
Mean	9.95	10.94	2.24	3.07	0.23	0.61

Comparison of the roots cultured with two culturing systems shows that MRL and RDW values of ZYQ8 in NS were respectively 79% and 78% of its counterpart in NNS while the two values of JX17 were both 89%. For RSR, the values in both ZYQ8 and JX17 decreased significantly during the nutrient supply with the individual ratio of 38% and 35% only. In the DH population, the means of the three parameters in NS were all decreased, corresponding to 91%, 73% and 38% of their counterparts in NNS, respectively. Individually, 94% of the DH lines presented a lower RDW value in NS while more than 70% lines decreased in the MRL value. For RSR, all lines expressed a lower value in NS which even decreased up to 3.7-fold for 4 lines. The peaks for the trait MRL and RDW in NNS are clearly separable from those in NS (Fig.1). For RSR, the value in NNS multiplied by a coefficient of 2.6 was almost overlapped with that in NS.

2.2 QTL mapping

In the nutrient system, a total of eight QTLs were identified for MRL (4), RDW (1), and RSR (3) on rice chromosome 2, 3, 4, 5, 6, 9 and 10, respectively (Table 2 and Fig.2). Five of them with plus additive values were from JX17 alleles [MRL (2), RDW (1), and RSR (2)] while the other three QTLs with minus additive values were contributed by ZYQ8 alleles [MRL (2), RDW (0), and RSR (1)]. Each single QTL could explain the phenotypic variance between 10.4% and 19.9%. In the non-nutrient system, five QTLs were identified on four rice
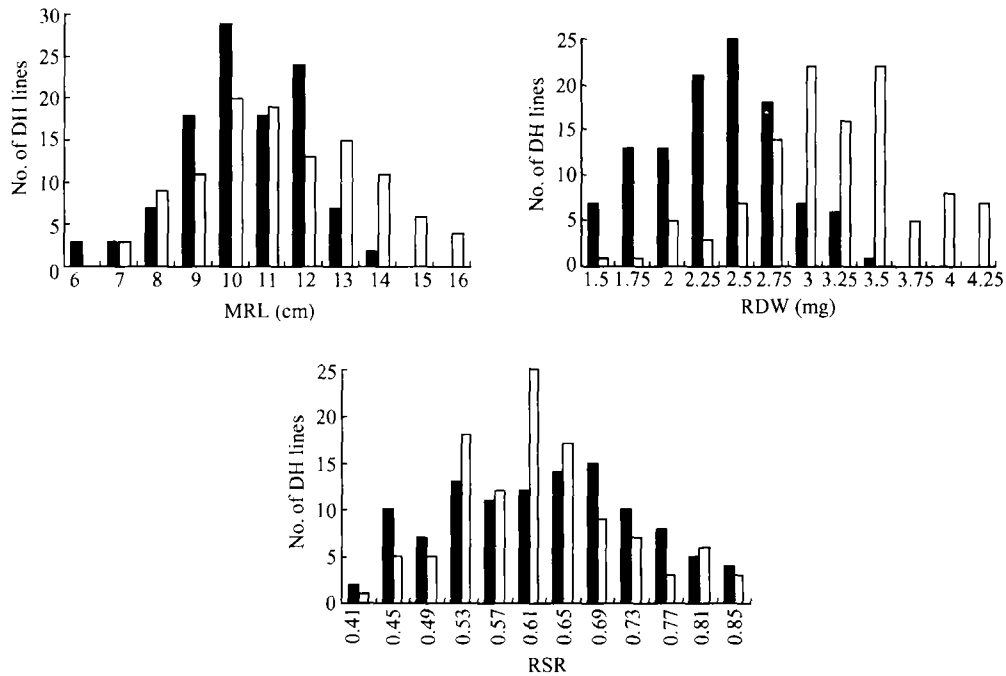
Fig. 1.   Frequency distribution of three root traits in DH population in two culturing systems. ■ in NS; □ in NNS.

chromosomes of 2, 3, 6 and 9. Four QTLs with plus additive value [MRL (1), RDW (1), and RSR (2)] were devoted by JX17 alleles. One QTL for RSR was from parent ZYQ8 allele. Each QTL could explain the phenotypic variation between 11.5% and 27.5%.

Table 2.   QTL mapping of the three rice root traits in nutrient (NS) or non-nutrient solution (NNS)

Traits	QTL	Marker interval	Chr.	LOD score	Variance (%)	Additive value
Nutrient solution:						
MRL	qMRL2	GA120~G357	2	4.02	16.4	1.4032
	qMRL4	G177~CT206	4	3.58	19.6	-1.5309
	qMRL9	C711~G103	9	3.43	17.0	1.4249
	qMRL10	L169~CT106A	10	2.98	13.0	-1.2673
RDW	qRDW2-1	G45~G1314A	2	3.89	16.4	0.3825
RSR	qRSR3-1	G62~G144	3	2.43	13.2	-0.0286
	qRSR5	RG573~C624	5	2.59	10.4	0.0278
	qRSR6	RG653~RG433	6	4.52	19.9	0.0355
Non-nutrient solution:						
MRL	qMRL6	RG433~G342	6	2.61	11.5	1.5515
RDW	qRDW2-2	GA285~CT580	2	3.77	19	5.5131
RSR	qRSR3-2	GA53~C746	3	4.03	22.2	-0.1074
	qRSR6	RG653~RG433	6	4.70	20.2	0.1030
	qRSR9	C711~G103	9	5.14	27.5	0.1190

Comparison of the QTLs placements under the two culturing systems shows that one QTL for RSR (qRSR6) located in the interval of "RG653 ~ RG433" on chromosome 6 was shared. And its linked marker RG433 was also associated with MRL in non-nutrient system. For RDW, qRDW2-1 in NS and qRDW-2 in NNS were on the same chromosome 2 but separated by a map distance of 19.3 cM. For RSR, "qRSR3-1" in NS and "qRSR3-2" in NNS were both located on the same chromosome 3 but with a distance of 20.6 cM apart. In addition, the interval of "C771-G103" on chromosome 9 contains two QTLs, one for MRL in NS and the other for RSR in NNS.
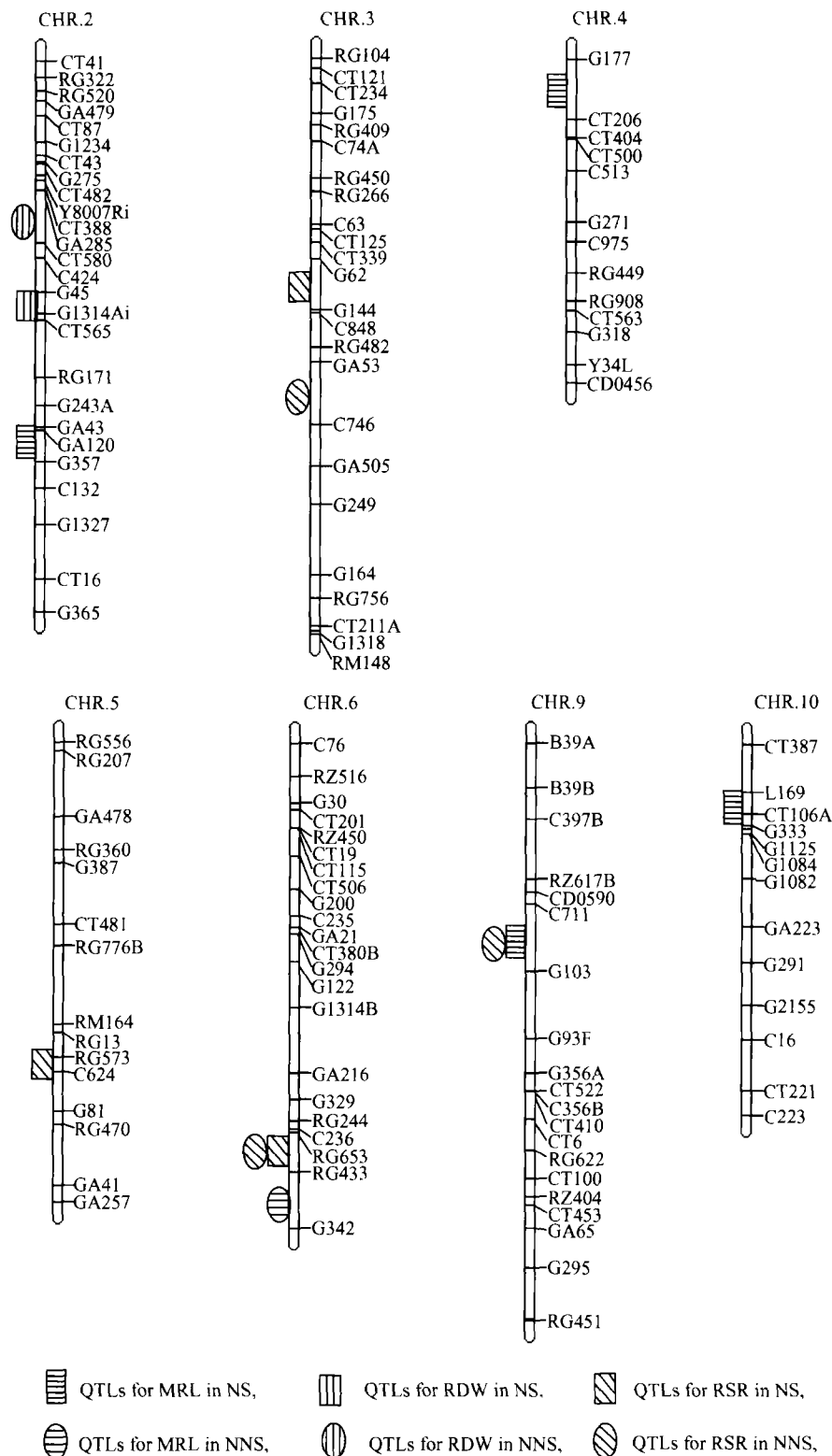
Fig. 2.   QTL mapping of rice root traits on chromosomes by the MAPMAKER/QTL program.

3   Discussions

In the previous reports, quantitative traits affected by environment factors were considered as variety specific and traits specific such as in maize[13], tomato[14], and soybean[15]. Of the three rice root

traits investigated in the present study, RSR was the most affected parameter that decreased in all the DH lines with an average of 2.6-fold due to the nutrient supply. The sensitivity to the nutrient supply also varied according to the individual line. The ratio of trait in NS to that in NNS was in a range of 46% ~ 162% for MRL, 42% ~ 138% for RDW, and 22% ~ 56% for RSR, respectively. The corresponding ones in the two parents, ZYQ8 and JX17, were separately 79% and 89% for MRL, 78% and 89% for RDW, 38% and 35% for RSR. It revealed that the *indica* type line as ZYQ8 displayed a little more sensitivity to the nutrient supply than the *japonica* type line as JX17.

In the comparable QTL mapping, eight and five QTLs were respectively identified related with the three root traits in NS and NNS. Only one QTL "qRSR6" was detected in both culturing systems while the others were culturing system specific. It was likely that some QTLs expressed in nutrient system but silenced in non-nutrient system while some QTLs expressed in non-nutrient system but silenced in nutrient system. In other words, the existence of over-amount of nutrient might restrain some QTLs expression such as "qMRL6", "qRDW2-2", "qRSR3-2", "qRSR9", whereas induce other QTLs expression of "qMRL2, qMRL4, qMRL9, qMRL10, qRDW2-1, qRSR3-1, qRSR5". It is obvious that QTLs functioned in terms of environment or environment specific, and the level of consistency in QTL mapping across environments was also trait specific, which is corresponding to its physiological character.

Among the 13 QTLs identified in this study, six QTLs were on chromosome 1, 2, 6 and 7 with a percentage of 46% while two QTLs were anchored on chromosome 5, 10, 11 and 12 with a percentage rate of 15% only. In comparison with the previous results[3,4,5,16], the corresponding rates are 48% and 17%, respectively. It distinguishably shows the uneven distribution of QTLs on chromosomes or chromosome specific.

The results here indicate that over-amount of nutrient would result in the lower phenotypic value such as MRL, RDW, and RSR, which are the important indices of rice drought resistance. So, appropriate lack of nutrient might be beneficial or valuable for the drought tolerance in the early vegetable stage of rice growth. Further efforts on systematic dissection of root gene expression could facilitate the understanding of the genetic and physiological mechanisms of the root traits, and also contribute the guidance to breeders in their breeding program.

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