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ORIGINAL PAPER

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QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid

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Abstract Tolerance to low nitrogen conditions is a highly desired characteristic for sustainable crop production. In this study, we analyzed the genetic components associated with low N tolerance in rice at seedling stage, including main effects, epistatic effects of the quantitative trait locus (QTLs), and QTL by environment interactions (QEs), using a population of 239 recombinant inbred lines (RILs) from a cross between Zhenshan 97 and Minghui 63, the parents of an elite hybrid. A genetic linkage map with 253 DNA maker loci was constructed. Seedlings of RILs were cultivated in low N and normal N solutions. Root, shoot and plant weight in the two N treatments were measured and the relative weight of the two treatments for each trait was considered as measurements for low N tolerance. Four to eight QTLs with main effects were detected for each of the nine traits. Very few QTLs were detected in both low and normal N conditions, and most QTLs for the relative measurements were different from those for traits under the two N treatments, indicating very little commonality in the genetic basis of the traits and their relative performance under low and normal N conditions. A total of 103 digenic interactions were detected for the nine traits. While the epistatic effects collectively accounted for large proportions of the variation for several traits, the effects of QEs appeared to be trivial. It was concluded that low N tolerance of rice seedling had complex genetic basis that requires extensive studies for full characterization.

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Introduction

Nitrogen is a crucial plant macronutrient that is needed in the greatest amount of all mineral elements required by plants. It comprises 1.5% to 2% of the plant dry matter and approximately 16% of total plant protein (Frink et al. 1999). In the last half a century, the global use of N fertilizer increased by approximately 10-fold in order to increase crop productivity (UNEP 1999), as a consequence of the fact that most of the high yielding varieties of the major crops developed in the last several decades have high demands of N and other nutrients. In general, plants consume much less than half of the fertilizers applied (Frink et al. 1999; Socolow 1999), while a majority of N fertilizers were lost to the atmosphere or leached into groundwater, lakes and rivers, which causes increasingly severe adverse effects to the environments.

Rice is the staple food for approximately half of the world's population. The proportion of N fertilizers lost is even higher in rice fields than in other cereal crops, because of rapid N losses from volatilization and denitrification in the soil-floodwater system (Vlek and Byrnes 1986). Loss of as much as 70% of the applied N fertilizers was reported in high yielding rice fields in China (Zhu 2000).

Additionally, fertilizer application has become a major economic cost for rice farmers especially in developing countries. Thus, developing crops that are less dependent on the heavy application of N fertilizers is essential for the sustainability of agriculture. Technically, this means development of crop varieties that can withstand soils of low N concentration by managing sufficient uptake (high uptake efficiency), and making best use of the N nutrient that the plant has absorbed from the soil for producing the products (high utilization efficiency).

N uptake and assimilation pathways in higher plants have been well documented. They involve a variety of transporters functioning to absorb the nutrients from

the soil (Crawford and Glass 1998; Forde 2000; Howitt and Udvardi 2000; Glass et al. 2001; Williams and Miller 2001), and a number of enzymes for assimilation and transfer of the absorbed N into amino acids and other compounds (Campbell 1988; Lam et al. 1996; Hirel and Lea 2001). However, little is known regarding how these elements and the processes are regulated especially in different N conditions.

Quantitative trait locus (QTL) analysis based on high density molecular linkage maps has become a powerful tool for dissecting the genetic basis underlying complex traits into individual components. Studies have been conducted in maize (Agrama et al. 1999; Bertin and Gallais 2000; Hirel et al. 2001; Gallais and Hirel 2004) and *Arabidopsis* (Loudet et al. 2003a, b) to identify QTLs governing various traits under low N stress and normal N conditions for characterization of the possible genetic factors regulating N metabolism. In maize, the results of Agrama et al. (1999) showed that some of the QTLs were detected under both low N stress and normal N conditions, while others were detected only by specific N treatments. However, the study of Bertin and Gallais (2001) showed that QTLs detected at normal N input were different from those detected under low N stress conditions. It was also reported that different N sources, such as nitrate, ammonium, ammonium and nitrate, or low N treatments, by which the studies were conducted, also lead to different results of QTL mapping in *Arabidopsis* (Rauh et al. 2002). In rice, QTL analyses have been conducted to map activities of the enzymes involved in ammonium assimilation (Yamaya et al. 2002; Obara et al. 2001, 2004), protein and nitrogen contents in flag leaves (Ishimaru et al. 2001) as well as plant height in different N levels (Fang and Wu 2001).

In the study reported in this paper, we analyzed main-effects and digenic interactions of QTLs for seedling growth under low N stress and normal N conditions using a recombinant inbred line (RIL) population derived from an elite rice hybrid. The goal was to identify QTLs for low N tolerance that might be useful for improving the N utilization efficiency of rice cultivars.

Materials and methods

Experimental population and phenotypic measurements

The population used in this study consisted of 239 F_{10} RILs derived by single-seed descent from a cross between two indica (*Oryza sativa* L. ssp. *indica*) lines, Zhenshan 97 and Minghui 63 (Xing et al. 2002), the parents of Shanyou 63, the most widely cultivated hybrid in China in the last two decades.

For phenotyping, 40 seeds for each of the RILs, the two parents and the F_1 were soaked in water at 10°C for 96 h and allowed to germinate at 35°C for 24 h. The germinated seeds were planted in sand with no nutrients. At the emergence of the second leaf, 14 seedlings per line were transplanted to a plastic box of 60 cm (length) × 36 cm (width) × 14 cm (height), containing 30 L of the culture solution (Yoshida et al. 1976), with a planting density of 7.0 cm by 9.5 cm. The plants were allowed to grow under natural conditions in Wuhan, China, with the cultural solution changed every three days during the course of cultivation. The whole set of materials was planted in duplicates. At the emergence of the fifth leaf, one set of the seedlings was transferred into a nutrient solution with the N concentration reduced to 0.24 mM NH_4NO_3 (one sixth of the normal N concentration) for low N stress, and the other set of the seedlings transferred to the normal N concentration solution. Two weeks later, the plants were harvested for trait scoring, with the roots and shoots separated. The harvested tissues were placed in a baker set at 110°C for 30 min followed by drying at 80°C for 4 days, after which the tissues were ready for taking measurements.

The entire planting experiment was replicated twice. The first replicate was raised from August 15 to September 20, 2003 and the second, from September 25 to November 10, 2003.

Trait measurements

Dry weight of shoots and roots was measured for each line. Relative shoot weight (RSW) was measured as the ratio of shoot weight under low N stress to the shoot weight under normal N control. Relative root weight (RRW) and relative plant weight (RPW) were similarly obtained as the corresponding ratios, which provide measurements for the degree of low tolerance for the genotypes tested.

DNA markers and map construction

A total of 253 polymorphic loci, including 168 RFLPs and 78 SSRs, were used to develop the genetic linkage map. Of them, 220 were from the previous work (Xing et al. 2002) and the other 33 SSRs were added to fill the gaps in the map. The RFLP marker assay followed the method described by Liu et al. (1997), and the SSR assay was conducted essentially as described by Wu and Tanksley (1993). A genetic linkage map was constructed using Mapmaker 3.0 (Lincoln et al. 1992).

Data analyses

QTL Mapper 1.6 (Wang et al. 1999) based on a mixed linear model approach (Zhu and Weir 1998) that estimates main-effect and digenic epistatic QTLs and predicts QE interaction effects simultaneously, was employed to analyze genetic components of the traits. In the analysis, likelihood ratio (LR) and *t* statistics were combined for testing hypotheses about QTL effects (including additive effects and digenic interactions) and QE interactions. Since replication appeared

to have significant effects on some of the traits (see Results), the two replications were treated as two environments in the QTL analysis to reveal possible QTL by environment interaction (QE) effects. Estimates of QTL effects (additive and epistasis) were obtained by the maximum-likelihood estimation method, while QE effects (additive by environment interactions and epistasis by environment interactions) were predicted using adjusted unbiased predictor. The LR value corresponding to $P = 0.005$ (equivalent to LOD = 4.03 for $df = 6$) was used as the threshold for claiming the presence of putative main-effect or epistatic QTLs. The significance of QTL effects, including additive effects, additive by additive epistatic effects, additive by environment interaction effects, and epistasis by environment interaction effects, was further tested by running the sub-menu of Bayesian test ($P < 0.005$). The peak points of the LR in the linkage map were taken as the putative positions of the effects. When a QTL was involved in more than one epistasis, its position and additive effect were taken from the point showing the largest effect. The relative contribution of a genetic component was calculated as the proportion of phenotypic variance explained by that component in the selected model, and the total contribution of the QTLs to the trait variation was calculated by adding up the percent contributions of individual QTLs.

Results

Measurements and variation of the traits

The results from analyses of variance (three-way ANOVA for the three traits, root weight, shoot weight and plant weight, and two-way ANOVA for the relative performance of the traits) are presented in Table 1. Among the three traits, N treatment accounted for the largest portions of the variation for shoot weight and plant weight, whereas genotype was the major source of variation for root weight. The effects of genotype by treatment interactions were highly significant for all three traits. In addition, the effect of replication was highly significant for shoot weight and plant weight, and significant for RRW. However, the effect of replication was not significant for root weight, RSW and RPW. The effects of replications were mostly due to differences in the environmental conditions in which the two replicates were implemented.

The measurements of the traits and their relative performance for the parents, F_1 and RILs are given in Table 2. There was a wide range of segregation for every trait investigated in the RIL population, suggesting that genes for these traits were highly dispersed in the two parents. The F_1 measurements of these traits were very close to the parents, suggesting that the gene actions were mostly additive.

Correlations between the traits

The coefficients of pairwise correlations between these traits are given in Table 3. As expected, the highest correlations were observed between shoot weight and plant weight, and between root weight and plant weight. The correlations were also high for RPW with RSW and RRW.

Table 3 also shows significant negative correlations between relative performance of each trait and its measurement under normal N conditions. For example, the correlation between RRW and root weight under normal N was −0.570, and those for shoot weight and plant weight were −0.631 and −0.638, respectively; all of them were significant at the 0.01 probability level, indicating a general trend that genotypes showing higher relative performance in the traits were smaller in size. However, exceptions were also obvious as can be seen from Fig. 1. There were RILs showing higher measurements than the parents and F_1, both in the relative performance and the scores under normal N conditions for shoot weight, root weight and plant weight.

Molecular-marker linkage map

The map consisting of 253 RFLP and SSR marker loci spanned a total of 1,678 cM in length with an average spacing of 6.6 cM between adjacent marker loci (Fig. 2). The length and the structure of the map are very similar to the one published previously using the same RIL population (Xing et al. 2002).

QTLs for root weight

For root weight under low N stress conditions (Table 4), seven main-effect QTLs were resolved, jointly explaining 30.6% of the phenotypic variation. Minghui 63 alleles at four of the QTLs, *n-r3*, *n-r4*, *n-r11a* and *n-r11b* were in the direction of increasing root weight, while the alleles from Zhenshan 97 at the other three QTLs, *n-r5*, *n-r9* and *n-r12* increased root weight. The QTL, *n-r5*, located in the interval R3166-RG360 of chromosome 5, had the largest effect by explaining 13.5% of the phenotypic variation.

Nine digenic interactions were detected for this trait involving 17 loci distributed on 10 of the chromosomes, accounting for 17.5% of the phenotypic variation (Table 4). Four of epistatic interactions involved main-effect QTLs. Parental two-locus genotypes for three of the nine pairs increased root weight, while recombinant two-locus combinations increased root weight for the other six pairs.

Significant environmental interactions were detected for *n-r4*, *n-r9*, *n-r11a* and *n-r11b* (Table 4), explaining 4.0% of the phenotype variation. No interaction was detected between the epistatic QTLs and the environments.

Table 1 ANOVA of root, shoot and plant weight in the RIL population under low N and normal N conditions

Trait	Source	*df*	SS	MS	*F*	*P*
Root weight	Genotype	238	54.44	0.2287	24.97	0
	Treatment	1	0.0002	0.0002	0.02	0.8799
	Replication	1	0.0238	0.0238	2.60	0.1074
	G×T	238	13.520	0.0568	6.20	0
	Error	477	4.369	0.0092		
Shoot weight	Genotype	238	491.88	2.0667	558.6	0
	Treatment	1	2197.30	2197.3	593906	0
	Replication	1	1.1426	1.1426	308.8	0
	G×T	238	221.38	0.9301	251.4	0
	Error	477	1.7648	0.0037		
Plant weight	Genotype	238	819.30	3.44	247.2	0
	Treatment	1	2195.9	2195.9	157680	0
	Replication	1	1.4964	1.4964	107.4	0
	G×T	238	308.64	1.2968	93.1	0
	Error	477	6.6429	0.019		
Relative root weight	Genotype	238	9.9166	0.0417	8. 8	0
	Replication	1	0.0270	0.0270	5.7	0.0176
	Error	238	1.1271	0.0047		
Relative shoot weight	Genotype	238	3.2028	0.0134	232.60	0
	Replication	1	0.00003	0.00003	0.48	0.4899
	Error	238	0.0138	0.00006		
Relative plant weight	Genotype	238	3.8534	0.0162	66.99	0
	Replication	1	0.0004	0.0004	1.48	0.2244
	Error	238	0.0575	0.0002		

Table 2 Absolute (in grams) and relative measurements of the root, shoot and plant weight for parents, hybrid and the RILs under low N and normal N conditions

Traits	Zhenshan 97	Minghui 63	F_1	RIL population	
				Mean	Range
N-RW	0.31	0.30	0.30	0.32	0.20–0.44
N+RW	0.27	0.28	0.29	0.32	0.19–0.51
RRW	1.13	1.05	1.03	1.00	0.66–1.38
N-SW	0.63	0.64	0.65	0.66	0.40–0.98
N+SW	1.17	1.25	1.28	1.26	0.77–2.13
RSW	0.54	0.51	0.51	0.52	0.35–0.75
N-PW	0.94	0.93	0.95	0.98	0.60–1.39
N+PW	1.44	1.53	1.56	1.59	1.00–2.64
RPW	0.65	0.61	0.61	0.62	0.41–0.87

N-RW root weight under low N stress conditions; *N+RW* root weight under normal N conditions; *RRW* relative root weight in two N treatments; *N-SW* shoot weight under low N stress conditions; *N+SW* shoot weight under normal N conditions; *RSW* relative shoot weight in two N treatments; *N-PW* plant weight under low N stress conditions; *N+PW* plant weight under normal N conditions; *RPW* relative plant weight in two N treatments

For root weight under normal N conditions (Table 5), five main-effect QTLs were detected, jointly explaining 11.4% of the phenotypic variation. Sixteen digenic interactions were resolved involving 30 loci distributed on all 12 chromosomes (Table 5), which accounted for 41.0% of the phenotypic variation in total. Significant environmental interactions were detected only for *n+r11* (Table 5), accounting for 0.7% of the phenotype variation.

Table 6 shows the QTLs for RRW in the two N treatments. Four QTLs showing main effects on the RRW were mapped on chromosomes 1, 7 and 11, which

Table 3 Correlation coefficients among the trait measurements in the RIL population

	N-RW	N+RW	RRW	N-SW	N+SW	RSW	N-PW	N+PW
N+RW	0.613							
RRW	0.283	−0.570						
N-SW	0.701	0.480	0.137					
N+SW	0.528	0.866	−0.509	0.536				
RSW	0.052	−0.521	0.708	0.295	−0.631			
N-PW	0.862	0.564	0.201	0.966	0.573	0.229		
N+PW	0.558	0.913	−0.533	0.536	0.994	−0.622	0.584	
RPW	0.147	−0.544	0.833	0.251	−0.644	0.977	0.232	−0.638

See footnotes of Table 2 for abbreviated names

$r_{0.05} = 0.138$; and $r_{0.01} = 0.181$

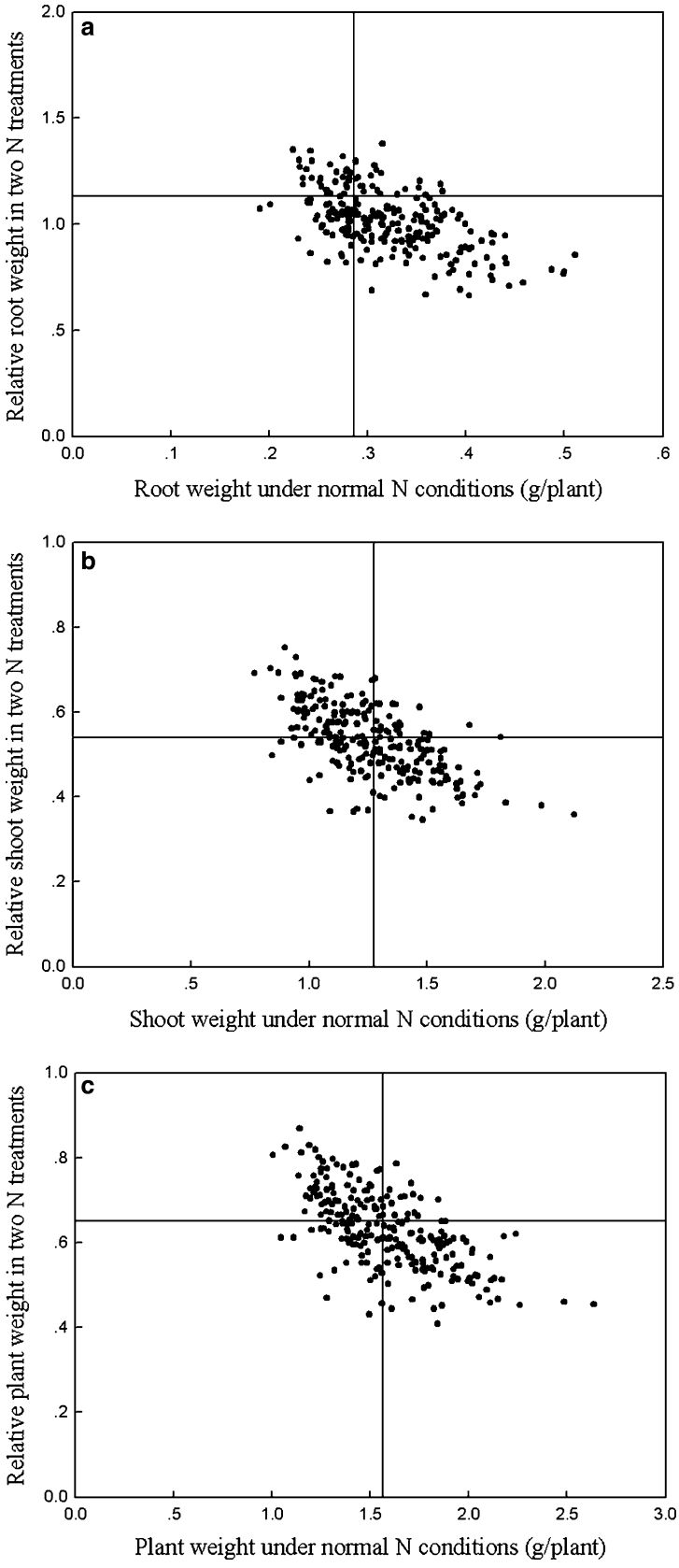

Fig. 1 Relationships of relative root, shoot and plant weight with root, shoot and plant weight under normal N conditions. The *horizontal* and *vertical lines* represent the highest values of the respective attributes among the two parents and F_1

jointly explained 16.7% of the phenotypic variation. One QTL, *rrw1b*, located in the interval C86-RG236 on chromosome 1, had a large effect on the trait by explaining 9.8% of the phenotypic variation. Twelve digenic interactions were detected for RWT involving 23 loci dispersed on 10 chromosomes, accounting for

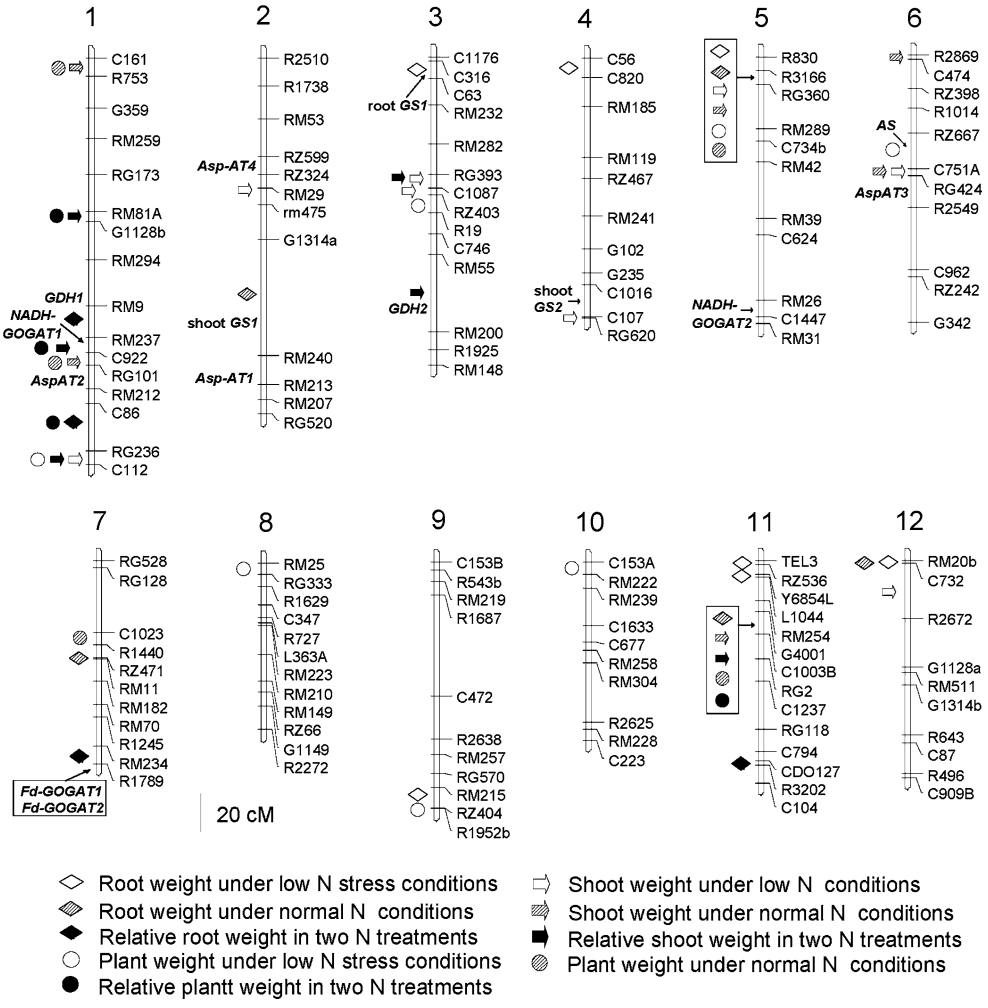

Fig. 2 Locations of the QTLs for root, shoot, plant weight and their relative measurements under low N and normal N conditions

Table 4 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for root weight under low N stress conditions with the LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i[b]	h^2a_i[e]	a_j[b]	h^2a_j[e]	aa_{ij}[c]	h^2aa_{ij}[e]	ae_j[d]	h^2ae_j[e]	h^2total[f]
1-2	R753-G359		5-1	R830-R3166		8.04					0.04	2.62			2.62
1-21	C2340-C86		11-17	RG103-CDO534		4.19					−0.03	1.70			1.70
2-7	R712-RZ324		5-2	R3166-RG360	*n-r5*	25.17			−0.09	13.5					13.48
2-7	R712-RZ324		12-6	RM179-C996		5.00					−0.04	2.13	0.02	0.22	2.35
2-18	RM208-RM207		4-1	C56-C820	*n-r4*	7.01			0.04	2.62			−0.06	1.60	4.22
3-2	C316-C63	*n-r3*	6-25	R2549-C962		6.85	0.03	1.14			−0.05	3.30			4.44
3-18	R1925-RM148		9-19	RM215-RZ404	*n-r9*	6.63			−0.02	0.90	−0.03	1.22	−0.05	1.05	3.17
4-4	C751B-RM185		11-9	Y6854L-L1044	*n-r11a*	10.14			0.04	2.62	0.02	0.75	0.04	0.56	3.93
5-5	C734b-RM42		11-1	TEL3-RZ536	*n-r11b*	9.82			0.05	3.44	−0.02	0.90	−0.05	0.82	5.16
5-15	C1447-RM31		12-1	RM20b-C732	*n-r12*	14.06			−0.06	6.38					6.38
8-17	G1149-R2272		9-15	RM242-RG570		4.33					−0.04	2.37			2.37
11-3	R543a-Y6855R		12-6	RM179-C996		5.88					0.04	2.49			2.49

General contributions: additive(A); $h^2(A) = 30.60\%$; epistasis: $h^2(AA) = 17.48\%$; QE interactions: $h^2(AE) = 4.25\%$

[a] Ch-Ini and Ch-Inj represent the chromosome number-interval of the points being tested in the analysis

[b] a_i and a_j are the additive effects of testing points i and j, respectively. Positive values of a_i and a_j imply that the Minghui 63 genotype taking a positive effect on that trait

[c] aa_{ij} is the effect of additive by additive interaction between points i and j; a positive value indicates that the parental two-locus genotypes have a positive effects and the recombinants had a negative effect

[d] ae_j is the effect of interaction between locus j and the environment; a negative value indicates that the effect in the first repeat is larger than the second repeat

[e] h^2a_i, h^2a_j, h^2aa_{ij} and h^2ae_j are the percentages of the phenotypic variations explained by a_i, a_j, aa_{ij}, ae_i and ae_j, respectively

[f] h^2total is the phenotypic variation explained by the genetic components included in the model

40.7% of the phenotypic variation in total. No significant interactions were detected between the QTLs (main-effect or epistatic) and environments.

QTLs for shoot weight

For shoot weight under low N stress conditions (Table 7), eight QTLs were resolved as showing main effects, collectively explaining 31.9% of the phenotypic variation. The QTL, *n-s5*, located in the interval R3166-RG360 of chromosome 5, had the largest effect explaining 8.8% of the phenotypic variation. Eleven digenic interactions were detected to account for 17.8% of the phenotypic variation, involving 20 loci distributed on 11 chromosomes. Significant environmental interactions were detected for *n-s3b* and *n-s6*, but only accounting for 0.02% of the phenotype variation.

For shoot weight under normal N conditions (Table 8), six QTLs showing main effects on shoot weight were detected, jointly accounting for 21.9% of the phenotypic variation. Eight digenic interactions were detected for this trait involving 14 loci on 10 chromosomes, which accounted for 24.6% of the phenotypic variation. No significant interactions were detected between the QTLs and environments.

Table 9 presents the QTLs for RSW in two N treatments. Six QTLs showing main effects on RSW were detected, which explained 25.0% of the phenotypic variation in total. Fourteen digenic interactions were detected for this trait involving 26 loci dispersed on nine chromosomes and accounting for 35.9% of the phenotypic variation. No significant environmental interactions were detected for all the QTLs.

QTLs for plant weight

For plant weight under low N stress conditions (Table 10), seven QTLs were detected as showing main effects, which jointly explained 22.5% of the phenotypic variation. The QTL, *n-p5*, located in the interval R3166-RG360 of chromosome 5, had the biggest contribution, explaining 9.7% of the phenotypic variation, with the allele from Zhenshan 97 contributing to the increase of this trait. Ten digenic interactions were resolved for this trait, involving 20 loci distributed on 10 chromosomes and accounting for 17.8% of the phenotypic variation collectively. No significant QE was detected.

For plant weight under normal N conditions (Table 11), the analysis resolved five main effect QTLs, which jointly explained 16.1% of the phenotypic variation. The 13 digenic interactions accounted for 38.3% of the phenotypic variation with none of them involving a main-effect QTL. Again, no significant QE was detected.

Table 12 shows results of QTL analysis for relative plant weight in two N treatments. Four QTLs were detected as showing main effects on RPW, collectively explaining 20.4% of the phenotypic variation. Ten digenic interactions were detected involving 20 loci dispersed on nine chromosomes, which accounted for 24.9% of the phenotypic variation in total. Again no QE was detected for this trait.

Relationship of the QTLs detected in these nine traits

For root weight, two QTLs were common between the seven QTLs detected under low N stress and five QTLs resolved under normal N conditions (Fig. 2, Table. 4, 5). Whereas, none of the four QTLs resolved for RRW

Table 5 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for root weight under normal N conditions with the LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	ae_j^d	$h^2ae_j^e$	h^2total[f]
1-1	C161-R753		3-4	rm251-RM232		6.63					−0.05	2.86			2.86
1-12	RM294-RM9		10-14	C371-C405a		4.27					0.04	2.02			2.02
1-21	C2340-C86		6-7	C688-R1952a		5.50					0.05	2.42			2.42
2-15	G1314a-RM240	*n+r2*	6-26	C962-RZ242		6.52	0.04	1.65			0.05	3.22			4.87
3-4	rm251-RM232		8-17	G1149-R2272		5.26					−0.05	2.53			2.53
3-5	RM232-RM282		11-9	Y6854L-L1044		4.47					−0.04	1.48			1.48
3-15	RM55-RM200		6-22	RZ667-C751A		5.97					−0.05	3.09			3.09
4-1	C56-C820		6-28	RG653-G342		4.53					−0.05	2.64			2.64
5-1	R830-R3166		6-28	RG653-G342		6.85					−0.06	3.72			3.72
5-2	R3166-RG360	*n+r5*	6-9	R2749-C1368		9.97	−0.07	5.14							5.14
5-6	RM42-RM39		11-29	CDO127-R3202		5.51					0.06	3.98			3.98
5-14	RM26-C1447		10-15	C405a-C223		5.63					0.05	2.86			2.86
5-15	C1447-RM31		12-1	RM20b-C732	*n+r12*	6.34			−0.04	1.48	−0.04	2.02			3.50
6-4	C952-Waxy		9-13	R2638-RM257		4.08					−0.04	2.12			2.12
6-11	R1962-C764		11-15	G4001-C1003B	*n+r11*	7.47			0.04	1.83	0.04	1.57	−0.05	0.66	4.06
7-5	RG678-RZ471	*n+r7*	12-6	RM179-C996		4.81	−0.03	1.32			−0.04	1.48			2.80
7-9	RM336-RM70		10-5	C148-RM239		5.37					0.05	2.98			2.98

General contributions: additive(A): $h^2(A) = 11.42\%$; epistasis: $h^2(AA) = 40.99\%$; QE interactions: $h^2(AE) = 0.66\%$

[a–f] See footnotes of Table 4 for explanations

Table 6 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for relative root weight in two N treatments with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	ae_j^d	$h^2ae_j^e$	h^2total[f]
1-7	RG173-RM81A		10-6	RM239-C1633		6.20					−0.03	3.70			3.70
1-13	RM9-RM5		3-5	RM232-RM282		4.34					0.02	2.24			2.24
1-14	RM5-RM237	*rrw1a*	7-12	RM234-R1789	*rrw7*	9.85	−0.03	3.70	−0.02	2.24					5.94
1-22	C86-RG236	*rrw1b*	12-9	G1314b-R643		15.37	−0.04	9.82							9.82
2-15	G1314a-RM240		11-19	RM21-RG2		4.03					0.02	2.45			2.45
2-20	RM48-RG520		9-12	C472-R2638		4.45					−0.02	2.03	−0.01	0.05	2.08
3-15	RM55-RM200		4-12	G235-R78		4.08					−0.02	2.03			2.03
4-5	RM185-RM119		11-8	C405b-Y6854L		5.68					0.03	4.87			4.87
7-6	RZ471-RM11		11-29	CDO127-R3202	*rrw11*	4.58			0.01	0.99	0.02	1.64			2.63
7-11	R1245-RM234		12-7	C996-RM511		6.56					0.03	3.70	0.01	0.05	3.75
8-7	C347-RG978		11-19	RM21-RG2		6.79					0.03	3.97			3.97
8-11	R727-L363A		11-28	R2918-CDO127		8.74					−0.02	2.68			2.68
9-1	C153B-C2		9-10	RM219-R1687		4.28					−0.03	3.70			3.70
9-17	RM201-RG667		12-2	C732-R2672		8.09					0.04	7.71			7.71

General contributions: additive(A): $h^2(A) = 16.75\%$; epistasis: $h^2(AA) = 40.72\%$; QE interactions: $h^2(AE) = 0.1\%$
[a–f] See footnotes of Table 4 for explanations

Table 7 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for shoot weight under low N stress conditions with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	ae_j^d	$h^2ae_j^e$	h^2total[f]
1-4	RG532-RM259		1-20	RM212-C2340		4.05					0.06	1.45			1.45
1-6	RM243-RG173		10-15	C405a-C223		4.80					−0.06	1.32			1.32
1-23	RG236-C112	*n-s1*	2-20	RM48-RG520		7.33	−0.09	2.87			0.05	0.85			3.72
2-9	RM29-R1843	*n-s2*	7-4	R1440-RG678		4.90	0.07	1.79							1.79
3-2	C316-C63		12-2	C732-R2672	*n-s12*	9.36			−0.07	1.89	−0.07	1.84			3.73
3-2	C316-C63		6-28	RG653-G342		4.25					−0.07	1.84			1.84
3-8	RG393-C1087	*n-s3a*	6-11	R1962-C764		13.54	0.14	6.75							6.75
3-9	C1087-RZ403	*n-s3b*	7-3	C1023-R1440		9.65	0.10	3.33					−0.01	0.01	3.34
4-1	C56-C820		11-8	C405b-Y6854L		5.05					0.07	1.79			1.79
4-14	C1016-C107		5-2	R3166-RG360	*n-s5*	22.57			−0.16	8.85					8.85
4-14	C1016-C107		6-3	R3139-C952		4.71					−0.08	1.99			1.99
4-15	C107-RG620	*n-s4*	11-12	Y2668L-G389		10.56	−0.10	3.69							3.69
5-15	C1447-RM31		11-30	R3202-RM20a		8.63					−0.09	2.93			2.93
6-14	RM204-C226		9-15	RM242-RG570		6.09					−0.06	1.19			1.19
6-23	C751A-RG424	*n-s6*	12-9	G1314b-R643		12.74	0.09	2.74			0.06	1.36	−0.01	0.01	4.11
6-28	RG653-G342		7-5	RG678-RZ471		4.34					−0.06	1.28	−0.04	0.13	1.41

General contributions: additive(A): $h^2(A) = 31.91\%$; epistasis: $h^2(AA) = 17.84\%$; QE interactions: $h^2(AE) = 0.15\%$
[a–f] See footnotes of Table 4 for explanations

Table 8 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for shoot weight under normal N conditions with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	h^2total[f]
1-1	C161-R753	*n + s1a*	12-2	C732-R2672		9.87	−0.27	4.49					4.49
1-16	C922-RG101	*n + s1b*	4-13	R78-C1016		7.45	0.24	3.63					3.63
2-3	RG634-R1738		4-13	R78-C1016		6.58					−0.21	2.75	2.75
2-12	RM341-RZ386		3-15	RM55-RM200		6.46					−0.19	2.20	2.20
2-17	RM213-RM208		6-1	R2869-C474	*n + s6b*	4.50			−0.20	2.49			2.49
3-1	C1176-C316		11-15	G4001-C1003B	*n + s11*	8.35			0.24	3.48			3.48
3-15	RM55-RM200		8-12	L363A-RM223		5.08					0.20	2.54	2.54
4-8	C2807-RM241		9-1	C153B-C2		7.78					−0.25	4.06	4.06
4-13	R78-C1016		6-14	RM204-C226		4.30					−0.18	1.95	1.95
5-2	R3166-RG360	*n + s5*	6-26	C962-RZ242		7.25	−0.24	3.48					3.48
5-13	C246-RM26		7-12	RM234-R1789		5.31					−0.25	3.78	3.78
6-16	RZ398-R1014		12-2	C732-R2672		5.22					−0.18	2.11	2.11
6-23	C751A-RG424	*n + s6a*	10-2	RM222-R2174		8.11	0.26	4.36					4.36
11-22	RM209-G257		11-30	R3202-RM20a		9.03					−0.29	5.26	5.26

General contributions: additive(A): $h^2(A) = 21.93\%$; epistasis: $h^2(AA) = 24.65\%$; QE interactions: $h^2(AE) = 0$
[a–c, e, f] See footnotes of Table 4 for explanations

Table 9 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for relative shoot weight in two N treatments with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	ae_j^d	$h^2ae_j^e$	h^2total[f]
1-2	R753-G359		3-18	R1925-RM148		5.06					−0.01	2.11			2.11
1-1	C161-R753		5-14	RM26-C1447		5.29					−0.01	2.11	−0.01	0.01	2.12
1-2	R753-G359		3-6	RM282-G144		4.93					0.01	2.45			2.45
1-8	RM81A-G1128b	*rsw1a*	2-8	RZ324-RM29		12.89	0.02	6.05							6.05
1-15	RM237-C922	*rsw1b*	8-17	G1149-R2272		19.70	−0.02	6.62			0.01	2.11			8.73
1-23	RG236-C112	*rsw1c*	7-8	RM182-RM336		11.00	−0.02	6.05							6.05
3-5	RM232-RM282		8-17	G1149-R2272		6.91					0.02	3.20			3.20
3-8	RG393-C1087	*rsw3a*	6-26	C962-RZ242		10.27	0.01	2.11			−0.01	1.51			3.62
3-15	RM55-RM200	*rsw3b*	6-22	RZ667-C751A		9.31	0.01	1.01			0.02	6.05			7.06
4-1	C56-C820		6-28	RG653-G342		6.63					0.01	2.45			2.45
5-6	RM42-RM39		11-15	G4001-C1003B	*rsw11*	10.17			−0.02	3.20	0.01	1.25			4.45
5-13	C246-RM26		8-12	L363A-RM223		4.14					0.01	1.80			1.80
6-4	C952-Waxy		12-6	RM179-C996		5.24					−0.01	1.51			1.51
6-14	RM204-C226		11-12	Y2668L-G389		9.38					−0.02	4.05	0.01	0.01	4.06
8-6	C483-C347		11-20	RG2-RM229		5.83					0.01	2.45			2.45
10-12	RG561-RM228		11-7	RM224-C405b		6.73					−0.02	2.81			2.81

General contributions: additive(A): $h^2(A) = 25.04\%$; epistasis: $h^2(AA) = 35.86\%$; QE interactions: $h^2(AE) = 0.02\%$
[a–f] See footnotes of Table 4 for explanations

Table 10 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for plant weight under low N stress conditions with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	h^2total[f]
1-1	C161-R753		7-7	RM11-RM182		5.44					0.11	1.97	1.97
1-19	R2201-RM212		2-20	RM48-RG520		4.53					0.12	2.11	2.11
1-23	RG236-C112	*n-p1*	11-17	RG103-CDO534		13.07	−0.12	2.15			−0.12	2.30	4.45
2-14	rm475-G1314a		3-10	RZ403-R19	*n-p3*	15.23			0.19	5.95			5.95
2-18	RM208-RM207		3-1	C1176-C316		4.27					0.08	1.13	1.13
4-1	C56-C820		11-9	Y6854L-L1044		4.82					0.09	1.24	1.24
4-9	RM241-G102		10-1	C153A-RM222	*n-p10*	5.10			−0.07	0.80	0.11	1.79	2.59
5-2	R3166-RG360	*n-p5*	10-11	R2625-RG561		24.90	−0.25	9.74					9.74
5-6	RM42-RM39		6-22	RZ667-C751A	*n-p6*	7.26			0.13	2.70			2.70
5-15	C1447-RM31		11-30	R3202-RM20a		6.81					−0.12	2.42	2.42
7-3	C1023-R1440		9-20	RZ404-R1952b	*n-p9*	5.53			−0.06	0.61	−0.09	1.15	1.76
8-1	RM25-RG333	*n-p8*	11-25	C1237-RG118		6.93	−0.06	0.52			0.17	2.53	3.05
9-15	RM242-RG570		10-7	C1633-C677		6.83					−0.09	1.15	1.15

General contributions: additive(A): $h^2(A) = 22.47\%$; epistasis: $h^2(AA) = 17.79\%$; QE interactions: $h^2(AE) = 0$
[a–c, e, f] See footnotes of Table 4 for explanations

Table 11 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for plant weight under normal N conditions with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i^b	$h^2a_i^e$	a_j^b	$h^2a_j^e$	aa_{ij}^c	$h^2aa_{ij}^e$	h^2total[f]
1-1	C161-R753	*n+p1a*	4-1	C56-C820		8.07	−0.28	3.32					3.32
1-16	C922-RG101	*n+p1b*	7-3	C1023-R1440	*n+p7*	10.26	0.24	2.36	−0.21	1.85			4.21
1-21	C2340-C86		3-18	R1925-RM148		4.48					0.21	1.81	1.81
2-11	C777-RM341		7-8	RM182-RM336		6.19					−0.23	2.16	2.16
2-12	RM341-RZ386		3-15	RM55-RM200		6.78					−0.26	2.82	2.82
3-15	RM55-RM200		6-22	RZ667-C751A		8.63					−0.35	5.03	5.03
3-18	R1925-RM148		11-9	Y6854L-L1044		9.22					−0.31	3.93	3.93
4-8	C2807-RM241		9-1	C153B-C2		6.20					−0.25	2.73	2.73
5-2	R3166-RG360	*n+p5*	8-4	C1121-R1629		9.98	−0.30	3.91					3.91
5-11	C624-RM274		6-20	Y4073L-G200		6.45					0.29	3.56	3.56
5-14	RM26-C1447		10-15	C405a-C223		6.9					0.21	1.92	1.92
7-2	RG128-C1023		12-7	C996-RM511		5.11					−0.19	1.56	1.56
7-9	RM336-RM70		10-1	C153A-RM222		5.44					0.25	2.60	2.60
8-1	RM25-RG333		11-25	C1237-RG118		7.45					0.35	5.03	5.03
8-1	RM25-RG333		10-10	RM304-R2625		5.53					0.25	2.58	2.58
9-18	RG667-RM215		11-15	G4001-C1003B	*n+p11*	10.89			0.33	4.69			4.69
11-21	RM229-RM209		11-30	R3202-RM20a		4.64					−0.25	2.54	2.54

General contributions: additive(A): $h^2(A) = 16.13\%$; epistasis: $h^2(AA) = 38.27\%$; QE interactions: $h^2(AE) = 0$
[a–c, e, f] See footnotes of Table 4 for explanations

was the same as those for root weight under either low N or normal N conditions.

Similarly, for shoot weight, two QTLs were common between the eight QTLs detected under low N and six QTLs under normal N conditions. Two of the QTLs for RSW (*rsw1c* and *rsw3a*) were common with QTLs for shoot weight under low N stress in the same directions, and one (*rsw11*) common with QTLs under normal N conditions but in opposite directions.

For plant weight, one QTL was common between the seven QTLs detected under low N stress and five QTLs under normal N conditions. One QTL for RPW (*rpw11*) was common with a QTL for plant weight under normal N conditions, but in opposite directions.

There were several QTL hotspots for these traits (Fig. 2). One of them was located on the short arm of chromosome 5, where six QTLs for both root and shoot weight under both low N and normal N conditions were resolved. Apparently this represents an important location for plant growth irrespective of the growth conditions. The second hotspot is located in the short arm of chromosome 11, which has a relatively large effect on five of the traits. Interestingly, most QTLs for relative measurements of the traits are located on chromosome 1, with a few others on chromosomes 11, 3 and 7.

Discussion

In this study, we partitioned the genetic basis of seedling growth at the seedling stage under two different N treatment conditions into main-effects, digenic epistatic effects, and QTL by environment interactions. A general feature that emerged from this analysis is that the QTL main effects were in general small, as evaluated by LOD scores and the amounts of variation explained, compared with main effect QTLs for yield and quality traits (e.g. Xing et al. 2002; Tan et al. 1999, 2001) identified in the same population. In contrast, the relative importance of epistatic effects is more pronounced for these traits than for yield and quality traits. The amounts of variation explained by epistatic effects were much larger than the amounts due to main effects for root, shoot and plant weight under normal N conditions, which is also the case for relative weight of root, shoot and plant. The overall effects of QEs are trivial, given the experimental conditions of relatively uniform cultural solutions.

The analysis showed that the QTLs for the traits detected separately in two different N treatments were mostly different, although certain commonalities existed among the three attributes of each trait as reflected by the QTL hotspots. Such different QTLs suggested that growth of root and shoot in different N conditions was regulated by different sets of genes, similar to the results obtained in studies of other plant species (Agrama et al. 1999; Bertin and Gallais 2000; Hirel et al. 2001; Loudet et al. 2003).

The most important outcome of this study resulted from mapping of the relative weight of root, shoot and plant under two different N treatments, which can be regarded as tolerance of the genotypes to low N stress. The analysis showed that most of the QTLs for relative performance were different from those for root, shoot and plant weight detected under the two N treatment conditions. Thus, the genetic basis of the relative performance cannot simply be deduced on the basis of separate detections of QTLs under different N treatments, as was done in all the previous studies. It is also interesting that the distribution of QTLs for the relative performance is concentrated on chromosome 1, which should be targeted for identifying genes of this nature in future studies.

It should be noted that the locations of some QTLs seem to correspond to loci for N assimilation and transfer deduced on the basis of genomic sequences (Fig. 2). For example, *NADH-GOGAT1* was located in a region where QTLs for RPW (*rpw1a*, chr 1), and RSW (*rsw1b*, chr 1) were detected in both N treatments. Root *GS1* was located in the vicinity of a QTL for root weight under low N stress conditions (*n-r3*, chr 3), and *GDH2* corresponded to a region where a QTL for RSW (*rsw3b*, chr 3) was detected in both N treatments. In addition,

Table 12 Main effects, epistatic effects and environment interactions of QTLs identified using QTLMapper 1.6 for relative plant weight in two N treatments with a LOD threshold 4.03 (equivalent to a chi-square value at $P = 0.005$ for $df = 6$)

Ch-Ini[a]	Flanking markers	QTL	Ch-Inj[a]	Flanking markers	QTL	LOD	a_i[b]	h^2a_i[e]	a_j[b]	h^2a_j[e]	aa_{ij}[c]	h^2aa_{ij}[e]	ae_j[d]	h^2ae_j[e]	h^2total[f]
1-1	C161-R753		11-15	G4001-C1003B	*rpw11*	7.71			−0.02	4.62					4.62
1-8	RM81A-G1128b	*rpw1b*	4-4	C751B-RM185		6.33	0.01	0.96			0.01	1.16			2.12
1-15	RM237-C922	*rpw1a*	6-15	C226-RZ398		8.70	−0.02	5.05							5.05
1-17	RG101-G393		8-3	R902-C1121		7.06					0.02	2.45			2.45
1-21	C2340-C86		4-15	C107-RG620		4.93					0.02	2.15			2.15
1-22	C86-RG236	*rpw1c*	6-18	RZ588-R2147		19.53	−0.03	9.78			0.02	2.15			11.93
3-3	C63-rm251		8-1	RM25-RG333		6.93					0.02	3.10			3.10
3-5	RM232-RM282		8-17	G1149-R2272		7.02					0.02	3.82			3.82
4-3	C933-C751B		10-14	C371-C405a		4.88					0.01	1.38			1.38
5-13	C246-RM26		8-12	L363A-RM223		7.31					0.02	3.45			3.45
6-14	RM204-C226		11-12	Y2668L-G389		5.55					−0.02	2.45	0.01	0.24	2.69
8-7	C347-RG978		11-19	RM21-RG2		5.24					0.02	2.76			2.76

General contributions: additive(A): $h^2(A) = 20.41\%$; epistasis: $h^2(AA) = 24.87\%$; QE interactions: $h^2(AE) = 0.48\%$

[a–f] See footnotes of Table 4 for explanations

the location of *AS* was in the region of a QTL for plant weight (*n-p6*, chr 6) under low N stress conditions. These results may be helpful for gene identification using a candidate gene approach.

Modern cultivars have been bred for high yield under high input conditions including heavy application of N fertilizers. The large increase of fertilizer application as a common agricultural practice in many countries has greatly increased environmental pollution, accompanied by largely reduced rate of fertilizer utilization by the crops. For sustainable production of crops like rice, cultivars that can maintain the productivity level at reduced fertilizer application are crucial. In this connection, it should be noted that there are high negative correlations of RRW, RSW and RPW with the weights of the respective attributes under normal N conditions, indicating a general trend that genotypes with smaller plant size had higher relative values of the traits. Thus, genotypes with small plant size apparently suffered less from low N stress than the ones with big size, due to the limited N supply in the cultural solution. However, there were also exceptions as indicated by a number of RILs that produced high trait values and also showed relatively high values of the relative performance compared with the parents and the F_1. These RILs may be explored further for identification of genotypes of high N use efficiency.

What needs to be further investigated is how the performance of the traits, especially the relative measurements observed in the cultural solutions, were related to performance under field conditions. It should also be noted that root weight, shoot weight, plant weight and their relative performance under the two N conditions investigated in this study reflected the total effects of uptake and utilization. The relative contributions of the two components, and the physiological processes in N metabolism underlying the QTLs have yet to be established in future studies. Thus, this work is only a starting point for characterizing the genetic basis of rice growth in different N levels, while many studies are necessary for fully understanding the biological mechanisms of nitrogen uptake and the utilization efficiency under relative low N conditions.

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