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Identification of quantitative trait loci for grain yield and its components in response to low nitrogen application in rice

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Abstract

Increasing grain yield with high nitrogen (N) use efficiency and less N application is an important objective in rice genetic improvement. A total of 127 rice recombinant inbred lines derived from Zhenshan 97 × Minghui 63 (*Oryza sativa* L.) were used for detecting the quantitative trait loci (QTLs) of grain yield and its components (panicle per m^2, spikelets per panicle, grain filling ratio, and 1000-grain weight) under low (LN) and normal nitrogen (NN) applications in 2006 and 2007. Among 68 QTLs detected across the two years under LN and NN, 33 QTLs were identified under LN (18 in 2006 and 15 in 2007), and 35 QTLs under NN (18 in 2006 and 17 in 2007). Only ten of 33 QTLs (30.3%) under LN and 14 of 35 QTLs (40%) under NN were detected simultaneously in both two years. A total of 18 of 36 QTLs (50%) in 2006 and 16 of 32 QTLs (50%) in 2007 were simultaneously detected under the two N applications, respectively. The results showed that environments over the two years and N applications had their significant effects of QTL expressions. The seven regions (RG532-G359-R753 on chromosome 1, RM53-R1738 on chromosome 2, RZ403-C1087- RG393 on chromosome 3, RM26-C246-C624 and C734b-RG360 on chromosome 5, R1440-C1023 and RZ471-RG678 on chromosome 7) were found to have their effects simultaneously under LN and NN in a year or two years. Eight regions were only detected under LN. Our results may provide information for genetically improving rice NUE by keeping yield stability when decreasing N application, and by increasing yield under current level of N application. Those regions that are stable across various nitrogen applications and responsive to LN would be useful for improving grain yield formation with high N use efficiency and less N application in rice breeding.

Keywords*:* Grain yield; Yield components; Recombinant inbred lines; Quantitative trait loci; Low nitrogen application.
Abbreviations: LN - low nitrogen application; NN – normal nitrogen application; NUE - nitrogen use efficiency; QTLs-Quantitative trait loci; RILs- recombinant inbred lines.

Introduction

Nitrogen (N) is one of the most important nutrients for the rice plant (Yoshida.1981), increasing N use efficiency (NUE) and reducing N application are of significance for sustainable rice production. Therefore, Zhang et al. (2007) brought forward that high nutrient efficiency and great reduction in chemical fertilizers should be the characteristics of green super rice. Although NUE has a large variation among various genotypes and is affected by growth environments, selecting genotypes with a stable NUE is still the proper way for improving NUE (Singh et al., 1998; Inthapanya et al., 2000). In previous studies, some traits related to NUE could be considered for the improvement of NUE. For instance, Cho et al. (2007) suggested that grain yield as an indirect selection index under low nitrogen (LN) was used for the improvement of NUE because higher grain yield could result in higher NUE under LN. Ju et al. (2006) also reported that NUE was positively correlated with grain yield, grain filling ratio and spikelets per panicle in two years. From the correlated traits of a breeding perspective, grain yield and its components under different nitrogen conditions could be considered as indirect selection criteria for the breeding of improved NUE in rice. Detection of quantitative trait locus (QTL) has become a tool for dissecting the genetic bases of complex traits such as NUE, grain yield and its components. QTL mapping for yield related traits has been conducted extensively under normal nitrogen (NN) application. For rice, more than 2000 QTLs for the yield-related traits have been reported (Gramene, www.gramene.org). Among those QTLs, a few QTLs were however located under LN. Cho et al., (2007) identified two grain yield QTLs in the RILs of Dasanbyeo/TR22183 under LN. Senapathy et al. (2008) detected three QTLs for grain yield, panicles per m^2, and grain filling ratio under LN, and also found that these QTLs had strong interactions with the amount of nitrogen fertilizer. Tong et al. (2011) reported 19, 23 and 15 QTLs detected for grain yield and its components under low, normal, and high N conditions, respectively. Only a few QTLs

for the same trait were simultaneously detected at three nitrogen levels, showing that QTLs were sensitive to N applications and different environments. Similarly, the relationships between grain yield and its components under different N conditions need to be further analyzed at the QTL level, which might provide useful information for improving NUE in rice breeding. The objectives of this study are to identify the QTLs affecting grain yield and its components under LN and NN, to further understand the responses of yield formation to nitrogen applications at the QTL level in rice.

Results

Phenotypic variations

In the two years under LN and NN, MH63 was significantly different from ZS97 for all traits except panicles per m^2 in 2006, as shown in Table 1. For the two parents and lines in both years, all traits except spikelets per panicle in 2007 and 1000-grain weight showed significant differences between LN and NN. The analysis of variance indicated that effects of genotype, N and year had significant differences for all traits (Table 2). The year had more effects on the five traits than N application. Except for genotype-by-nitrogen interaction of grain yield, genotype-by-nitrogen and genotype-by-year interactions had significant effects on all traits. The broad-sense heritabilities over the two years ranged from the lowest value of 0.40 for grain filling ratio under NN to the highest value of 0.96 for 1000-grain weight under LN (Table 2). In comparison with grain filling ratio and spikelets per panicle, the other traits (1000-grain weight, grain yield, panicles per m^2) had higher heritabilities under LN and NN, respectively. Over the two N applications, high heritabilities for all traits except grain filling ratio and spikelets per panicle in 2006 were observed, ranging from 0.86 for panicles per m^2 in 2007 to 0.98 for 1000-grain weight in 2007.

Correlations among the traits

The table 3 showed the correlations among grain yield and its component traits. Generally, phenotypic correlations among traits were similar as genetic correlations, regardless of the degree of correlation. It was noteworthy that significant phenotypic correlations of grain yield with panicles per m^2 and as well with 1000-grain weight were observed under LN and NN in both years, there were however no genetic correlations between them except that between grain yield and 1000-grain weight under NN in 2007. In 2006, grain yield had the highest phenotypic and genetic correlations with grain filling ratio, and the second highest ones with spikelet per panicle under both N conditions. Under LN and NN in 2007, the highest correlations were between grain yield and spikelets per panicle, followed by the second highest ones between grain yield and grain filling ratio. Those results suggested that contributions of the two yield components to grain yield may be different over the years.

QTL detection

Grain yield

Under LN, five QTLs for grain yield (qGY*l*2-1, qGY*l*2-2, qGY*l*2-3, qGY*l*7-1 and qGY*l*7-2) were identified on chromosomes 2 and 7 in 2006, which individually explained the phenotypic variation by 7.6% to 12.19%. Only two QTLs were detected on chromosomes 7 and 11 in 2007, contributing 22.48% of the total phenotypic variation (Table 4 and Fig 1). Under NN, four QTLs (qGYn2-1, qGYn7-1, qGYn7-2 and qGYn7-3) were detected on chromosomes 2 and 7 in 2006, accounting for 67.18% of the total phenotypic variation. The QTL qGYn7-3 in the genomic region C1023-RG128 showed the largest additive effect and contributed 24.43% of the phenotypic variation. Three QTLs (qGYn1, qGYn2-2 and qGYn7-4) were identified on chromosomes 1, 2 and 7 in 2007, contributing 31.91% of the total phenotypic variation (Table 4 and Fig 1). Summarily, the region RM53-R1738 on chromosome 2 was detected for grain yield (qGY*l*2-3, qGYn2-1 and qGYn2-2) under both LN and NN in 2006 and under NN in 2007. These QTLs overlapped, and all alleles at the loci increasing the phenotypic variations were from ZS97. The region RZ471-RG678 on chromosome 7 was also identified for four grain yield QTLs (qGY*l*7-1, qGY*l*7-3, qGYn7-2 and qGYn7-4) under LN and NN in both years, and the alleles from MH63 increased the phenotypic values.

Grain filling ratio

Under LN, three QTLs for grain filling ratio (qGF*l*1, qGF*l*3-1 and qGF*l*7) were identified on chromosomes 1, 3 and 7 in 2006, which collectively contributed 55.85% of the total phenotypic variation. The qGF*l*7 on the region C1023-RG128 of chromosome 7 showed the largest effect and accounted for 30.58% of the total variation (Table 4 and Fig 1). Two major QTLs (qGF*l*3-2 and qGF*l*4) were located on chromosomes 3 and 4 in 2007, totally accounting for 32.4% of the variation. The qGF*l*3-2 in the region RZ403-C1087 had the largest effect, accounting for 20.51% of the total phenotypic variation.
Under NN, two QTLs (qGFn2-1 and qGFn7) in 2006 and three QTLs (qGFn1, qGFn2-2 and qGFn3) in 2007 were positioned on chromosomes 1, 2, 3 and 7, respectively. Collectively, 23.25% and 38.33% of the total phenotypic variation were explained in both years (Table 4 and Fig 1). The region of RZ403-C1087 on chromosome 3 was simultaneously shared by the two QTLs (qGF*l*3-2 and qGFn3) under LN and NN in 2007.

Spikelets per panicle

Under LN, two QTLs (qSPP*l*3 and qSPP*l*5) were identified on chromosomes 3 and 5 in 2006, which explained 20.61% of the total phenotypic variation. Four QTLs (qSSP*l*1, qSSP*l*2, qSSP*l*7-1 and qSSP*l*7-2) were located on chromosomes 1, 2 and 7 in 2007, and the phenotypic variation explained by the individual QTLs ranged from 7.02% to 10.36% (Table 4 and Fig 1). Under NN, two QTLs in 2006 (qSPPn1 and qSPPn5) and two QTLs in 2007 (qSPPn2 and qSPPn7) were identified on chromosomes 1, 2, 5 and 7, which contributed 21.59% and 20.03% of the total phenotypic variation in both years (Table 4 and Fig 1), respectively. The qSPP*l*7-2 under LN and qSPPn7 under NN were detected on the same region R1440-C1023 of chromosome 7 in 2007.

Panicles per m^2

Under LN, three QTLs (qPPM*l*3, qPPM*l*12-1 and qPPM*l*12-2) in 2006 and two QTLs in 2007 (qPPM*l*1 and qPPM*l*7) were positioned on chromosomes 1, 3, 7 and 12, which contributed 33.76% of the total phenotypic variation in 2006 and 20.53% in 2007 (Table 4 and Fig 1), respectively. Under NN, four QTLs in 2006 (qPPMn1-1, qPPMn5, qPPMn7-1 and qPPMn7-2) and three QTLs in 2007 (qPPMn1-2, qPPMn7-3 and qPPMn11) were identified on chromosomes 1, 5, 7 and 11. The identified QTLs explained 45.89% of the variation in 2006 and 26.81% in 2007 (Table 4 and Fig 1). The genomic region R1440-C1023

Table 1. Performances of grain yield and its components in parents and RILs derived from a cross between ZS97/MH63 under low (LN) and normal (NN) nitrogen applications in the two years

Traits	N level	MH63 [a]	ZS97 [a]	LSD [b]	Mean [a]	RILs Range	Skew	Kurt
2006								
GY	LN	6.69 ± 0.17	4.18 ± 0.29	*	5.49 ± 0.73	2.67 - 7.41	-0.30	1.72
	NN	7.76 ± 0.49	4.97 ± 0.12	***	6.44 ± 0.80	4.18 - 8.86	-0.12	0.36
GF	LN	82.81± 2.74	75.33 ± 0.92	*	83.8 ± 5.6	70.7 - 94.5	-0.38	-0.72
	NN	74.81 ± 1.72	77.58 ± 0.51	*	82.6 ± 7.2	54.1 - 93.4	-1.17	1.18
SPP	LN	92.03 ± 4.22	70.37 ± 6.98	*	90.3 ± 15.7	67.2 - 139.9	0.94	0.55
	NN	101.57 ± 3.78	74.66 ± 4.37	**	94.8 ± 16.7	58.3 - 171.9	0.47	0.63
PPM	LN	340.83 ± 14.22	340.17 ± 15.25	ns	307.9 ± 34.9	225.8 - 410.0	0.41	0.47
	NN	390.00 ± 11.46	397.50 ± 13.23	ns	354.7 ± 39.5	252.5 - 479.2	0.48	0.51
TGW	LN	26.49 ± 0.45^{ns}	23.10 ± 0.52	**	24.7 ± 2.6	18.3 - 30.3	0.23	-0.43
	NN	26.18 ± 0.48	21.63 ± 0.96	**	24.0 ± 2.2	18.2 - 29.9	0.13	-0.26
2007								
GY	LN	5.63 ± 0.36	2.94 ± 0.37	***	4.50 ± 0.79	1.79 - 5.98	-0.80	0.79
	NN	6.37 ± 0.20	3.63 ± 0.11	***	5.34 ± 0.77	2.84 - 6.83	-0.71	0.63
GF	LN	76.62 ± 2.42	83.08 ± 1.49	*	76.7 ± 8.8	42.9 - 90.1	-0.85	1.03
	NN	77.61 ± 2.71	67.28 ± 2.57	**	72.9 ± 8.2	47.5 - 89.4	-0.61	0.19
SPP	LN	103.50 ± 7.54^{ns}	75.43 ± 2.55^{ns}	**	107.3 ± 23.3 ^{ns}	50.9 - 192.1	0.68	1.16
	NN	103.65 ± 11.09	83.26 ± 0.64	*	105.9 ± 22.0	58.4 - 172.9	0.70	0.96
PPM	LN	245.00 ± 25.0	194.17 ± 15.26	*	211.7 ± 25.6	138.3 - 302.7	0.41	1.26
	NN	295.00 ± 6.61	270.83 ± 8.78	*	266.0 ± 34.6	192.5 - 362.5	0.50	0.33
TGW	LN	27.36 ± 0.68^{ns}	24.09 ± 0.44^{ns}	**	25.6 ± 2.8 ^{ns}	18.8 - 31.4	0.11	-0.72
	NN	27.29 ± 0.37	23.90 ± 0.54	***	25.5 ± 2.9	19.1 - 31.2	0.11	-0.74

GY, grain yield (t ha^{-1}); GF, grain filling ratio (%); SPP, spikelets per panicle; PPM, panicles per m^2; TGW, 1000-grain weight (g). [a]: value without ns (not significant) indicate significant difference for between LN and NN at P≤0.05 by least significant difference (LSD) for the identical parent and by ANOVA for RI lines. [b]: ***,**, and * indicated the significant differences under the same nitrogen application between the parents at ≤0.001, 0.01, and 0.05, respectively; ns, not significant.

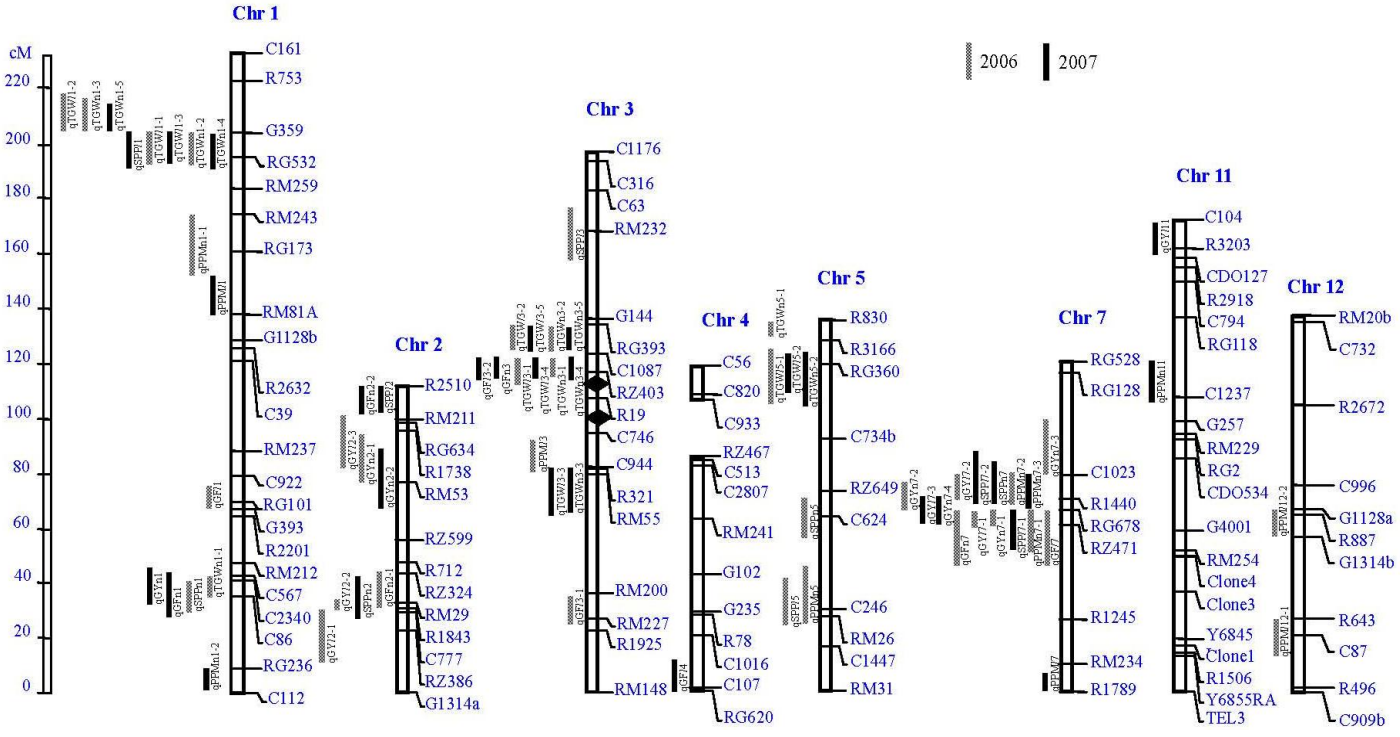

Fig 1. Genetic linkage map showing the location of QTLs for the traits investigated in the ZS97/MH63 RIL population.

on chromosome 7 was detected for qPPMn7-2 under NN in 2006, which also harbored qPPMn7-3 under NN in 2007.

1000-grain weight

Under LN, five QTLs were identified on chromosomes 1, 3 and 5 in 2006, with phenotypic variation explained by individual QTL ranging from 11.86% to 20.98%, and together accounting for 79.79% of the total phenotypic variation (Table 4 and Fig 1). Two QTLs (qTGW*l*1-1 and qTGW*l*1-2) had larger effects than the other QTLs and explained more than 20.00% of the total variation. Five QTLs (qTGW*l*1-3, qTGW*l*3-3, qTGW*l*3-4, qTGW*l*3-5 and qTGW*l*5-2) were located on chromosomes 1, 3 and 5 in 2007, with 79.32% of the total phenotypic variation explained, varying from 9.92% to 30.11%. The QTL qTGW*l*5-2 in C734b-RG360 on chromosome 5 had the largest effect and accounted for 30.11% of the total phenotypic variation in 2007. The QTL qTGW*l*1-3 in the region RG532-G359 had the second largest effect and accounted for 18.07% of the total phenotypic variation. Under NN, a total of six QTLs were detected on chromosomes 1, 3 and 5 in 2006 (Table 4 and Fig 1), collectively contributed 81.50% of the total phenotypic variation. Variation explained by the individual QTLs ranged from 7.18% to 17.21%. Three QTLs (qTGWn1-2, qTGWn3-1 and qTGWn3-2) had larger effects than the other QTLs and each explained more than 15% of the total phenotypic variation. Six QTLs were identified on chromosomes 1, 3 and 5 in 2007, which contributed 83.4% of the total phenotypic variation (Table 4 and Fig 1). The QTL qTGWn5-2 in the region C734b-RG360 of chromosome 5 had the largest effect and accounted for 27.15% of the total variation. Comparing QTLs detected under LN and NN, four QTLs (qTGW*l*1-2, qTGW*l*1-3, qTGWn1-2 and qTGWn1-4) simultaneously shared the same region (RG359-G359 of chromosome 1) under both N conditions in both years, and the alleles increasing phenotypic variation were from ZS97. The region RZ403-RG393 on chromosome 3 harbored eight QTLs under both N conditions in two years, at which the alleles increasing phenotypic variation were from MH63. The interval C734b-RG360 on chromosome 5 was also detected for two QTLs (qTGW*l*5-2 and qTGWn5-2) under both nitrogen conditions in 2007 and one QTL (qTGW*l*5-1) under LN in 2006. The alleles increasing phenotypic values were from ZS97.

Discussion

Effects of environments on QTLs for grain yield and its components

It is documented that environments have large effects on crop yield formation. In the study, effects of two N applications and two years were investigated. Firstly, environments over the two years had effects on grain yield formation. Among 33 QTLs under LN, only ten of 33 QTLs (30.3%, two for grain yield and eight for 1000-grain weight) detected simultaneously in both two years and involved in four intervals (RG532-G359 on chromosome 1, RZ403-C1087-RG393 on chromosome 3, C734b-RG360 on chromosome 5, and RZ471-RG678 on chromosome 7). Under NN, only 14 of 35 QTLs (40%, four for grain weight, two for panicle per m^2, and eight for 1000-grain weight) were found simultaneously in both two years and involved in four intervals (RG532-G359-R753 on chromosome 1, RZ599-RM53-R1738 on chromosome 2, RZ403-C1087-RG393 on chromosome 3, RZ471-RG678-R1440-C1023 on chromosome 7). Those results showed that environments over the two years had its significant effects of QTL expressions, especially for grain filling ratio, spikelets per panicle and panicles per m^2. It is noted that four and four QTLs for 1000-grain weight were detected simultaneously in both two years under LN and NN, respectively, suggesting the environmental stability across different years. Those results were consistent with the high heritabilities (H_B^2=0.96 under LN and 0.92 under NN) for 1000-grain weight, and as well with significant effects of year revealed by ANOVA (Table 2). Secondly, N applications affected the yield and its components as well. Among 36 QTLs in 2006, 18 QTLs (50%, eight for 1000-grain weight, six for grain yield, two for grain filling ratio, and two for spikelets per panicle) were detected under the two N applications, which involved in six intervals (RG532-G359-R753 on chromosome 1, RM53-R1738 on chromosome 2, RZ403-C1087- RG393 on chromosome 3, RM26-C246-C624 on chromosome 5, and R1245-RZ471-RG678 and R1440-C1023 on chromosome 7). Similarly in 2007, 16 of 32 QTLs (50%, ten for 1000-grain weight, two for grain yield, two for grain filling ratio, and two for spikelets per panicle) affected the yield related traits under both LN and NN, which located in six chromosomal regions (RG532-G359 in chromosome 1, RM200-RM55 and RZ403-C1087- RG393 in chromosome 3, C734b-RG360 on chromosome 5, R1440-C1023 and RZ471-RG678 on chromosome 7). Those results indicated that various N applications had their effects on yield formation and its QTL expressions. It was consistent with ANOVA, as shown in Table 2. On the other hand, at gene expression level, Lian et al. (2006) reported that 4.5% genes (471 of 10422) were detected as responsive to low N stress in the root tissue using a cDNA microarray. Similar results were observed in maize by Chen et al.(2011) and in soybean by Hao et al.(2011). For protein expression, Ding et al.(2011) identified 12 protein spots as responsive to LN. The differential expressions of QTLs to LN presented in the study are therefore in accord with those of expression profiles of genes and proteins. Summarily, four intervals (RZ471-RG678 for grain yield and RG532-G359, RZ403-C1087 and C1087-RG393 for 1000-grain weight) were simultaneously detected in the four environments (nitrogen application combining with year), three intervals (G359-R753 and C734b-RG360 for 1000-grain weight, G359-R753 for grain yield) were coexistent in three environments. However, most of candidate QTLs were detected in an environment, indicating that grain yield and its components were regulated by a number of different genes under different nitrogen conditions or different years. The observations matched with results of Tong et al. (2011). Interestingly, variances of environments across the two years had larger than those of nitrogen applications for the five traits (Table 2). Consistently, 50% QTLs (34/68) were detected simultaneously under both the two N applications, while only 35% (24/68) QTLs were detected simultaneously over the two years. So, the results presented in the paper showed that environments across the two years had more effects on yield related traits than those of nitrogen applications.

Comparisons of QTLs across different genetic backgrounds and over various environments

ZS97 and MH63 are the parents of Shanyou 63, the most widely grown rice hybrid in China. In various environments, several different genetic populations from the same parents ZS97 and MH63, including $F_{2:3}$ (Yu et al., 1997), immortalized F2 (IF2) (Hua et al., 2002), vegetatively replicated F_2 (VF_2) (Li et al., 2000), and RI populations (Xing et al., 2002; Guo et al., 2005), have been used in mapping yield-related traits in various

Table 2. Analysis of variance and broad sense heritability for grain yield and its components.

Source	df	Mean of square and significance level				
		GY	GF	SPP	PPM	TGW
Genotype (G)	126	5.99***	278.5***	2106***	10855***	79.64***
Nitrogen (N)	1	304.78***	2052***	969*	972033***	56.1***
Year (Y)	1	410.98***	27962***	75239***	3256328***	535.21***
G×N	126	0.19	81.2***	319***	891*	1.08*
G×Y	126	0.82***	287***	1904***	1472***	3.74***
Y×N	1	1.24**	474.1***	3242***	5425*	36.04***
G×Y×N	126	0.13^{ns}	76.7***	348***	707 ^{ns}	0.94 ^{ns}
Error	1016	0.16	29.4	200	703	0.79
Broad-sense heritability over the two years						
LN		0.85	0.50	0.49	0.78	0.96
NN		0.87	0.40	0.45	0.87	0.92
Broad-sense heritability over the two N applications						
2006		0.93	0.42	0.62	0.89	0.95
2007		0.96	0.91	0.94	0.86	0.98

GY, grain yield (t ha^{-1}); GF, grain filling ratio (%); SPP, spikelets per panicle; PPM, panicles per m^2; TGW, 1000-grain weight (g). ***,**, and * indicated the significant differences under the same nitrogen application between the parents at ⩽0.001, 0.01, and 0.05, respectively; ns, not significant.

Table 3. Phenotypic (upper) and genetic (lower) correlations among grain yield and its components in the RIL population under low (below the diagonal) and normal (above the diagonal) N applications.

Traits	GY	GF	SPP	PPM	TGW
2006					
GY		0.49***	0.31***	0.22*	0.23*
		0.49***	0.34***	0.04	0.04
GF	0.55***		-0.17*	-0.02	-0.24*
	0.57***		-0.17*	-0.02	-0.24*
SPP	0.26**	-0.05		-0.62***	-0.42***
	0.24*	-0.04		-0.63***	-0.44***
PPM	0.23*	-0.12	-0.56***		0.05
	0.12	-0.10	-0.61***		0.08
TGW	0.24*	-0.19*	-0.53***	0.10	
	0.08	-0.20*	-0.55***	0.12	
2007					
GY		0.26**	0.51***	0.20*	0.18*
		0.26**	0.53***	-0.07	0.18*
GF	0.35***		-0.29**	0.15	-0.31***
	0.35***		-0.29**	0.21*	-0.34***
SPP	0.54***	-0.23*		-0.58***	-0.08
	0.57***	-0.23*		-0.59***	-0.08
PPM	0.18*	0.08	-0.50***		-0.32***
	-0.07	0.11	-0.51***		-0.34***
TGW	0.20*	-0.27**	-0.13	-0.28**	
	0.14	-0.28**	-0.13	-0.32***	

GY, grain yield (t ha^{-1}); GF, grain filling ratio (%); SPP, spikelets per panicle; PPM, panicles per m^2; TGW, 1000-grain weight (g). * , ** and *** significant at P≤0.05, 0.01 and 0.001 probability levels, respectively.

environments. Multiple intervals related to the yield related traits in our study were also identified in previous reports. The region of C86-RM212 on chromosome 1 was detected for four QTLs (qGYn1, qGFn1, qSPPn1 and qTGWn1-1) under NN. This region was also detected for grain yield, 1000-grain weight, and panicles per m^2, and grain filling ratio in the same RI population by Xing et al. (2002) and Guo et al. (2005), for grain yield and 1000-grain weight in IF_2 by Hua et al.(2002). Those results suggest that the region for yield-related traits is environmental-independent, and could be used in improving yield formation. The region RG532-R753 on chromosome 1 was identified for 1000-grain weight under LN and NN across two years and spikelets per panicle under LN in 2007. This region was also found to control the two yield related traits (Yu et al., 1997; Hua et al., 2002; Xing et al., 2002) and panicles per m^2 (Yu et al., 1997) in the different populations from the same parents, ZS97 and MH63. The region R1738-R2510 on chromosome 2 were detected for grain filling ratio and grain yield under NN and for spikelets per panicle and grain yield under LN across two years, which was also identified for spikelets per panicle and panicles per m^2 by Yu et al. (1997) and Xing et al. (2002). The region RZ403-RG393 in chromosome 3 controlled two QTLs for grain filling ratio and eight QTLs for 1000-grain weight under LN and NN in both two years. This region was also identified for 1000-grain weight and spikelets per panicle in previous reports (Yu et al., 1997; Li et al., 2000; Hua et al., 2002; Xing et al., 2002). More interestingly, Fan et al. (2006) reported that *GS3* was located in this genomic region and exhibited the pleiotropic effects on both 1000-grain weight and grain length. The flanking region of marker C944 on chromosome 3 harbored QTLs for 1000-grain weight and panicles per m^2 in our study, which was also found to control 1000-grain weight (Yu et al., 1997; Xing et al., 2002; Hua et al., 2002). Similarly, Takeda et al. (2003) found that this genomic region contained the OsTB1 controlling rice tillering ability as a negative regulator for lateral branching. The region near RG360 on chromosome 5 was identified for 1000-grain weight under both nitrogen conditions in the study, which was also documented for grain yield and 1000-grain weight (Yu et al., 1997), for 1000-grain

weight and panicles per m^2 (Xing et al., 2002) and 1000-grain weight (Li et al., 2000; Hua et al., 2002) in the populations from the two parents. Similarly, Weng et al. (2008) reported that *GW5* gene increasing grain width as a result of enlarging cell number of the outer glume was found in this genomic region. The 15 QTLs for yield-related traits were clustered in the genomic region RZ471-C1023 of chromosome 7 in the study, showing major effects under both nitrogen conditions. This region was reported to host the QTLs for spikelets per panicle, panicles per m^2, grain yield, and 1000-grain weight in the various populations from ZS97 and MH63 (Xing et al., 2002; Guo et al., 2005; Yu et al., 1997; Li et al., 2000; Hua et al., 2002). The gene *Ghd7*, which had major effects on heading stage, plant height, grain number and grain yield, also were located on the region by Xue et al. (2008).

Relationship among grain yield related traits

Trait correlations are very prevalent phenomena, because complex traits do not develop independently but tend to be affected by other traits and different environments. For the genetic basis, pleiotropic effects and close linkage are often considered as the genetic basis for correlations (Chen and Lubberstedt, 2010). Regarding the relationships of grain yield with its components, grain yield had significant positive correlations with yield components at different N levels in our study (Table 3). It is same as common consideration that higher grain filling ratio, more spikelets per panicle, and more panicles per m^2 and larger 1000-grain weight favor to higher grain yield. This is supported by previous reports (Ju et al., 2006; Tong et al., 2011). In the study, several QTL clusters/co-locations for grain yield and its components were found. The first QTL cluster was located in the region C86-RM212 of chromosome 1, which affected the investigated yield traits except panicle per m^2 under NN, with all enhancing alleles from MH63 (Table 4, Fig 1). The contributions of these QTLs to trait performances were in the same direction. The second QTL cluster in the region R1843-RZ324 on chromosome 2 was identified for two grain yield QTLs under LN, and grain filling ratio and spikelets per panicle QTLs under NN. At the QTL cluster, the alleles increasing grain yield have the same parental contributor (ZS97) as those increasing the phenotypic scores of two yield components. At the two regions, alleles for grain yield have the same direction of additive effect as those for yield components, providing the base for the positive correlations between them. The co-locations were in accordance with the phenotypic and genetic correlations among the traits. The results suggest that simultaneous improvement of grain yield and its component is genetically feasible. Notably, the region RZ471-C1023 on chromosome 7 owned QTLs for the four yield-related traits but 1000-grain weight under both N applications in two years. At the region, ZS97 contributed alleles for increasing panicles per m^2, and MH63 provided alleles for increasing grain yield, grain filling ratio, and spikelets per panicle. This implies complexity of genetic constitution in the region, and fine mapping for specific detail is needed. On the other hand, negative correlations among yield components are wide spread and often observed in crop plants (Adams, 1967), as showed in the study (Table 3). At the three intervals (RM259-G359 on chromosome 1 for 1000-grain weight and spikelets per panicle, RM211-R2510 on chromosome 2 for grain filling ratio and spikelets per panicle, RM26-C246 on chromosome 5 for spikelets per panicle and panicles per m^2), alleles were from different parents, suggesting that parental contributions of the genetic effects to the controlled traits were in opposite directions, respectively. At the region RZ471-C1023 on chromosome 7 harbored QTLs for grain filling ratio, spikelets per panicle and panicles per m^2, and the alleles for increasing panicles per m^2 were contributed by ZS97, however, the alleles for other traits were from MH63. Those results reveal that it is in a dilemma for improving yield components simultaneously due to the yield component compensation. To unlock the undesirably negative correlations among yield components, gene linkage should be genetically broken. From the viewpoint of crop physiology, more assimilates distributed to filling spikelets, which might be approached by improving crop growth via adjusting tillering and as well proper applications of water and nutrients, might favor to relief the compensatory relationships among yield components. Overall, cluster and co-localization of the QTLs for various traits, as the results of either pleiotropic effects or close linkage, can provide an explanation for the genetic basis of correlations between the traits. However, it is noteworthy that co-localization or cluster of the QTLs may have occurred just by chance, due to the large number of the QTLs detected or large genetic distances between adjacent markers in this study.

Implications for breeding

Most of current rice varieties have been selected under the condition of high N fertilizer application for high grain yield. However, large N fertilizer application may result in severe adverse effects on the environments, yield formation and the poor eating and cooking quality of rice grains (Zhang, 2007). Developing variety without dependence on the heavy N application and with high NUE is essential for the sustainability of agriculture. NUE involves very complex physiological pathways closely associated with both yield formation and N accumulation, and is controlled by a large number of genes/QTLs, suggesting that NUE could be improved by selecting higher grain yield under LN. Tong et al. (2011) advanced that the identification of the genomic regions associated with yield related traits under LN and NN should be very useful for improving NUE by marker-assisted selection. Wei et al.(2012) also indicated nitrogen deficiency tolerance traits and performance under normal nitrogen condition should be simultaneously evaluated. Therefore, selecting for a stable genotype across different N applications might be a better strategy than exploring specific adaptation. In the study, the majority of QTLs for a given yield-related trait were different between LN and NN, suggesting that these QTLs were easily affected by different environments and were adaptive to specific environments (various N applications, various years). However, some co-localized or closely linked QTLs were still detected across LN and NN in the study. Overall, the study showed that several regions (RG532-G359-R753 and C86-RM212 on chromosome 1, RM53-R1738 on chromosome 2, RZ403-C1087-RG393 in chromosome 3, RM26-C246-C624 and C734b-RG360 on chromosome 5, R1440-C1023 and RZ471-RG678 on chromosome 7) were stable for grain yield and its components across the LN and NN. Additionally, eight chromosomal regions (G1314a-RZ386 on chromosome 2 and R3203- C104 on chromosome 11 for grain yield, G393-C922 on chromosome 1, RM227-RM200 on chromosome 3 and C107-C1016 on chromosome 4 for grain filling ratio, R1789-RM234 on chromosome 7, C87-R643 and G1314b-R887 on chromosome 12 for panicles per m^2) were only found to control yield and its components under LN, showing specific expressions only under LN. Our results may provide information for genetically improving rice NUE by

Table 4. Putative QTLs for grain yield and its components in four environments (nitrogen-by-year combinations) identified by composite interval mapping

N Application -Trait [a]	Year	QTL	Chr.	Interval	Peak (cM)	CI (cM)[b]	LOD	A[c]	R^2%[d]
LN-GY	2006	qGY*l*2-1	2	G1314a-RZ386	18.0	8.3-28.4	3.11	-0.21	10.77
		qGY*l*2-2	2	R1843-RM29	30.1	28.4-31.8	2.98	-0.18	7.60
		qGY*l*2-3	2	RM53-R1738	91.5	80.3-98.0	3.49	-0.20	10.25
		qGY*l*7-1	7	RZ471-RG678	62.0	59.6-65.0	4.00	0.22	12.16
		qGY*l*7-2	7	R1440-C1023	71.8	69.5-78.0	4.27	0.22	12.19
	2007	qGY*l*7-3	7	RZ471-RG678	64.0	60.1-70.8	4.32	0.29	13.10
		qGY*l*11	11	R3203-C104	162.7	156.3-169.2	2.89	0.24	9.38
NN-GY	2006	qGYn2-1	2	RM53-R1738	82.5	73.5-90.3	5.13	-0.34	18.53
		qGYn7-1	7	RZ471-RG678	63.0	59.8-65.9	3.84	0.27	11.34
		qGYn7-2	7	RG678-R1440	68.9	65.9-75.2	4.51	0.29	12.88
		qGYn7-3	7	C1023-RG128	90.0	78.0-99.2	3.18	0.40	24.43
	2007	qGYn1	1	C86-C2340	40.0	32.6-45.1	3.14	0.23	9.03
		qGYn2-2	2	RZ599-RM53	68.5	55.2-85.1	3.20	-0.26	12.01
		qGYn7-4	7	RZ471-RG678	65.0	60.1-70.8	3.80	0.25	10.87
LN-GF	2006	qGF*l*1	1	G393-C922	68.8	65.9-74.1	3.41	1.83	10.01
		qGF*l*3-1	3	RM227-RM200	27.8	25.1-34.1	4.58	-2.20	15.26
		qGF*l*7	7	R1245-RZ471	56.5	43.3-65.6	3.36	2.22	30.58
	2007	qGF*l*3-2	3	RZ403-C1087	116.7	112.7-120.1	7.16	-3.88	20.51
		qGF*l*4	4	C107-C1016	6.3	0.0-13.2	3.08	2.80	11.89
NN-GF	2006	qGFn2-1	2	RM29-RZ324	35.1	30.1-42.1	3.13	2.40	10.45
		qGFn7	7	R1245-RZ471	56.5	43.3-65.6	3.33	2.70	12.80
	2007	qGFn1	1	C86-C2340	37.0	26.2-42.6	3.48	2.52	10.14
		qGFn2-2	2	RM211-R2510	105.1	100.2-109.1	4.25	3.07	14.76
		qGFn3	3	RZ403-C1087	117.7	113.5-121.4	4.45	-2.95	13.43
LN-SPP	2006	qSPP*l*3	3	RM232-C63	166.2	153.7-173.6	2.82	-4.45	8.85
		qSPP*l*5	5	RM26-C246	26.8	22.0-40.4	3.83	5.18	11.76
	2007	qSPP*l*1	1	RG532-G359	192.6	185.2-201.3	2.81	6.23	7.02
		qSPP*l*2	2	RM211-R2510	108.1	98.8-109.1	3.28	-7.05	9.30
		qSPP*l*7-1	7	RZ471-RG678	63.0	50.6-65.9	3.01	6.85	8.69
		qSPP*l*7-2	7	R1440-C1023	70.8	67.3-87.6	3.87	7.54	10.36
NN-SPP	2006	qSPPn1	1	C86-C2340	35.0	29.5-40.7	3.65	6.31	11.33
		qSPPn5	5	C246-C624	62.8	52.0-68.0	3.31	-5.38	10.26
	2007	qSPPn2	2	R1843-RM29	30.1	25.7-39.1	3.84	-6.35	8.67
		qSPPn7	7	R1440-C1023	69.8	67.3-82.3	4.97	7.32	11.36
LN-PPM	2006	qPPM*l*3	3	C944-C746	84.6	79.4-91.3	4.48	12.94	14.02
		qPPM*l*12-1	12	C87-R643	20.9	13.0-26.7	3.64	-10.95	9.73
		qPPM*l*12-2	12	G1314b-R887	62.0	56.1-66.1	3.17	11.15	10.01
	2007	qPPM*l*1	1	RM18A-RG173	135.8	134.9-145.9	3.53	-10.56	11.28
		qPPM*l*7	7	R1789-RM234	0.0	0.0-7.0	3.06	-7.87	9.25
NN-PPM	2006	qPPMn1-1	1	RG173-RM243	160.7	145.8-171.2	3.51	-13.60	11.13
		qPPMn5	5	RM26-C246	26.8	22.5-46.6	3.63	-14.87	12.71
		qPPMn7-1	7	RZ471-RG678	63.0	52.3-65.9	2.99	-12.97	10.12
		qPPMn7-2	7	R1440-C1023	69.8	67.4-78.0	4.30	-13.96	11.93
	2007	qPPMn1-2	1	C112-RG236	3.0	0-8.4	3.21	10.34	8.90
		qPPMn7-3	7	R1440-C1023	69.8	65.9-78.0	3.39	-10.89	9.52
		qPPMn11	11	C1237-RG118	106.7	106.5-126.1	3.21	-10.12	8.39
LN-TGW	2006	qTGW*l*1-1	1	RG532-G359	196.6	189.7-201.3	9.42	-1.21	20.98
		qTGW*l*1-2	1	G359-R753	205.3	201.3-215.2	8.37	-1.20	20.54
		qTGW*l*3-1	3	RZ403-C1087	116.7	109.8-121.1	5.83	1.04	12.76
		qTGW*l*3-2	3	C1087-RG393	128.1	122.7-132.3	5.12	1.02	11.86
		qTGW*l*5-1	5	C734b-RG360	110.5	99.8-121.8	4.81	-0.98	13.65
	2007	qTGW*l*1-3	1	RG532-G359	196.6	188.3-201.3	9.01	-1.24	18.07
		qTGW*l*3-3	3	RM200-RM55	75.9	62.2-80.6	4.93	0.99	10.75
		qTGW*l*3-4	3	RZ403-C1087	116.7	112.8-120.3	5.21	0.99	10.47
		qTGW*l*3-5	3	C1087-RG393	127.1	122.1-132.2	4.21	0.98	9.92
		qTGW*l*5-2	5	C734b-RG360	111.5	105.8-120.3	11.14	-1.60	30.11
NN-TGW	2006	qTGWn1-1	1	C86-C2340	38.0	34.8-42.1	4.96	0.76	10.84
		qTGWn1-2	1	RG532-G359	197.6	189.3-201.3	10.12	-1.20	15.52
		qTGWn1-3	1	G359-R753	205.3	201.3-213.1	9.87	-1.10	14.75
		qTGWn3-1	3	RZ403-C1087	116.7	113.5-120.9	10.58	1.14	17.21
		qTGWn3-2	3	C1087-RG393	126.1	122.1-131.9	8.50	1.10	16.00
		qTGWn5-1	5	R3166-R830	130.7	125.7-131.7	3.32	-0.62	7.18
	2007	qTGWn1-4	1	RG532-G359	196.6	185.9-200.6	7.34	-1.13	14.91
		qTGWn1-5	1	G359-R753	204.3	201.3-211.4	6.23	-1.11	14.37
		qTGWn3-3	3	RM200-RM55	79.1	64.2-80.7	4.91	0.89	8.31
		qTGWn3-4	3	RZ403-C1087	117.7	112.6-122.1	4.48	0.94	8.95
		qTGWn3-5	3	C1087-RG393	127.1	123.7-131.2	3.92	0.94	9.71
		qTGWn5-2	5	C734b-RG360	112.5	105.2-121.3	10.80	-1.53	27.15

[a] LN and NN, low and normal nitrogen applications; GY, grain yield (t ha^{-1}); GF, grain filling ratio (%); SPP, spikelets per panicle; PPM, panicles per m^2; TGW, 1000-grain weight (g).[b] CI indicates confidence interval. [c] Negative value of additive effect indicates that the allele from ZS97 increases the phenotypic score. [d] R^2% represents the relative contributions of the individual QTL to the trait

increasing yield under current level of N application and by keeping yield stability when decreasing N application. These regions that are stable for grain yield related traits across different N applications and detected under LN have potential use for improving grain yield formation with high NUE and less N application in rice breeding.

Materials and methods

Plant materials

A RIL population derived from the cross of Zhenshan 97(ZS97) × Minghui (MH63) by single-seed descent was developed by Xing et al. (2002). A total of 127 lines selected randomly from 241 RILs (F_{10} and F_{11}) plus the two parents (Zhenshan97, ZS97) and Minghui63, MH63), were used in the field experiments.

Field experiments

The field experiments were conducted using a randomized block design with three replications under two N conditions at Xushan village, Xiaogan city (30°56" N 113°54" E) in 2006 and Dajin town, Wuxue city (29°51" N 115°33" E) in 2007, Hubei province in China, as described by Wei et al. (2011). The seeds were sown on May 20 in 2006 and on May 18 in 2007. The seedlings were transplanted at a planting density of 20 cm×16.7 cm on June 10 in 2006 and on June 8 in 2007. Each line was planted with three seedlings per hill. The plot sizes were 5.65 m^2 in 2006 and 8.2 m^2 in 2007. The soil type was Gleyed paddy soil with the following properties: pH 5.37, organic matter of 22.76 g kg^{-1}, total N of 1.09 g kg^{-1}, available P of 9.15 mg kg^{-1} and available K of 107.01 mg kg^{-1} at Xushan village; and pH 5.01, organic matter of 30.06 g kg^{-1}, total N of 1.39 g kg^{-1}, available P of 5.92 mg kg^{-1} and available K of 46.30 mg kg^{-1} at Dajin town. For LN, no artificial nitrogen fertilizer was applied. For NN, 135 kg N ha^{-1} was applied with three splits in 2006: 54 kg ha^{-1} as basal, 40.5 kg ha^{-1} at 15 days after transplanting (DAT) and 40.5 kg ha^{-1} at 25 DAT. A total of 130 kg N $ha^{-1}$was split into the same three applications in 2007. Under the two conditions, potassium (100 kg K ha^{-1}) was applied in two splits (50 kg ha^{-1} as basal and 50 kg ha^{-1} at 25 DAT) , phosphorus (40 kg P ha^{-1}) and zinc (5 kg Zn ha^{-1}) were applied as basal, respectively. All fertilizers were applied by hand broadcasting. Field management and chemical applications for disease and pest control were implemented to avoid yield loss during the whole growth duration.

Sampling and measurement of traits

Twelve hills for each line were cut at ground level and divided into leaf blades, stems plus leaf sheaths, and panicles at physiological maturity stage. Samples were oven-dried at 80 °C for 4 days and ground to powder by a grinder. About 0.2 g sample powder was used for analyzing nitrogen accumulation using a continuous-flow analyzer (Alliance, France) by standard micro-Kjeldal procedure (Yoshida et al. 1976). Twelve plants were sampled to determine yield components, including 1000-grain weight (g), grain filling ratio (%), spikelets per panicle, and panicles per m^2. Grain yield (t ha^{-1}) was determined by harvesting a 1-m^2 area from each plot.

Data analysis and QTL mapping

Analysis of variance was performed using the GLM procedure of SAS (SAS Inc, 1996). Considering each N application over the two years and as well each year over the two N applicaitons, broad-sense heritability (H_B^2) based on RIL means was estimated according to the following equation: $H_b^2=\sigma^2/(\sigma^2_g+\sigma^2_e/re+\sigma^2_{ge}/e)$, where σ^2_g is genetic variance, σ^2_e is residual variance, σ^2_{ge} is the variance of genotype × environment interaction, r is the number of replications per year (or N application), e is the number of environments (years or N applications). A linkage map was constructed by Xing et al. (2002). The map consisted of 168 RFLPs and 52 SSRs plus one morphological trait spanning a total of 1796 cM of the 12 rice chromosomes, with an average distance of 8.2 cM between adjacent markers. The composite interval mapping (CIM) method was performed for QTL analysis based on the genotypic means using WinQTLcart 2.0 software (Wang and Zeng, 2004). The walking speed chosen for all QTL analyses was 2 cM. A forward-backward step-wise multiple linear regression with a probability into and out of 0.05 and a window size of 10 cM was used to select the co-factor for controlling background effect. Logarithm of odds (LOD) for each trait was estimated from 1000 permutations (Churchill and Doerge, 1994). As a result of the permutation, the thresholds for declaring a QTL for the various traits ranged from 2.75 to 3.0. QTLs were named following the method described by McCouch et al. (1997).

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