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ORIGINAL PAPER

Mapping QTLs for yield and nitrogen-related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression

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Abstract

Key message **The present study identified some new important genomic regions and demonstrated the availability of conditional analysis in dissecting QTLs induced by environmental factors.**

Abstract The high input and low use efficiency of nutrient fertilizers require knowledge of the genetic control of crop reaction to nutrient supplements. In this study, 14 morphological and 8 physiological traits of a set of 182 wheat (*Triticum aestivum* L.) recombinant inbred lines (Xiaoyan 54 × Jing 411) were investigated in six environments to map quantitative trait loci (QTLs). The influence of nitrogen (N) and phosphorus (P) fertilization on QTL expression was studied by unconditional and conditional analysis. A total of 117 and 30 QTLs were detected by unconditional and conditional analysis, respectively, among which 21 were common for both methods. Thirty-four QTL clusters were identified. Eighteen conserved QTLs (15.4 % of the 117 QTLs) between years, but within nutritional treatment were found. The three major QTLs on chromosomes 2D, 4B and 6A were coincident with *Rht8*, *Rht-B1b* and *TaGW2*, respectively. The other two important intervals on chromosomes 4B and 7A for yield component traits were newly detected QTLs that warrant further study. By conditional analysis, spikelet number per spike was found to be induced by P fertilization mostly, whereas N fertilization had more effects on the expression of the QTLs for nitrogen concentration and utilization efficiency traits. QTLs that respond to N and P interactions were also detected. The results are helpful for understanding the genetic basis of N utilization efficiency in wheat under different N and P supplement environments and provide evidence for the availability of conditional analysis in dissecting QTLs induced by environmental factors.

Abbreviations

DM	Aboveground dry matter
DS	Drought stress treatment
FSS	Fertile spikelet number per spike
GNC	Grain nitrogen concentration
GNUP	Grain nitrogen uptake
GY	Grain yield
HI	Harvest index
KNS	Kernel number per spike
KWS	Kernel weight per spike
MAS	Marker-assisted selection
NHI	Nitrogen harvest index
NUP	Nitrogen uptake
$NUtE_{DM}$	N utilization efficiency for DM
$NUtE_{GY}$	N utilization efficiency for GY
PH	Plant height

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QTL	Quantitative trait locus
RIL	Recombinant inbred line
SCN	Spikelet compactness
SL	Spike length
SNC	Straw nitrogen concentration
SNP	Spike number per plant
SNUP	Straw nitrogen uptake
SSS	Sterile spikelet number per spike
SY	Straw yield
TKW	Thousand kernel weight
TSS	Total spikelet number per spike
WW	Well-watered treatment

Introduction

Nitrogen (N) is the most important mineral nutrient in crop growth and development. Large amounts of N fertilizers have been used to maximize crop yield in agricultural systems. Over the past four decades, the doubling of agricultural food production worldwide has been associated with a sevenfold increase in the use of N fertilizers (Hirel et al. 2007). The high input of N fertilizers resulted in low N use efficiency and caused a series of environmental and economical problems (Galloway et al. 2008; Hirel et al. 2007). Breeding new crop plant cultivars with improved productivity in low N environments provided an effective approach to increase N use efficiency (Rengel and Marschner 2005).

Wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is one of the world's most important cereal crops, which contributes approximately 30 % of the total cereal consumption in the world (FAO 2003). Considerable genetic variation for N use efficiency and related traits under different N levels has been reported in wheat by previous studies (Clárk 1983; Le Gouis et al. 2000; Wang et al. 2011), indicating a high likelihood of improving N use efficiency through a genetic approach. Quantitative trait locus (QTL) analysis has proved to be an effective approach to dissect a complicated quantitative trait into component loci to study their relative effects on the trait (Doerge 2002). Nowadays, QTL mapping has become a routine procedure for the identification of genomic regions harboring the genes which control polygenic traits (Saal et al. 2011). Several QTL experiments have been conducted in wheat to study N use efficiency under different N levels in hydroponic culture (An et al. 2006; Guo et al. 2012; Laperche et al. 2006), pot trails (Habash et al. 2007) and field trails (An et al. 2006; Fontaine et al. 2009; Laperche et al. 2007; Quarrie et al. 2005), or to identify QTLs for phosphorus (P) use efficiency in P sufficient and limited conditions (Li et al. 2007b; Su et al. 2006, 2009). In one of our previous studies, we reported QTLs for morphological, nutrient content and nutrient utilization efficiency traits grown at the seedling stage in diverse N, P and potassium (K) concentration combinations under hydroponic culture, and detected many important QTL clusters and cooperative uptake and utilization QTLs for N, P and K (Guo et al. 2012); while in another study, we identified QTLs for N uptake in field trails, and biomass production and N uptake in hydroponic culture under high and low N levels (An et al. 2006). However, studies on QTL analysis concerning nutrient interaction (i.e., N and P) at field trails are still needed to provide enough information to facilitate understanding the genetic basis of N uptake and utilization efficiency and the genetic improvement of wheat at different N or P supplements.

Conditional analysis can remove the variation due to component trait(s) and obtain the remaining variation (conditional variances) (Wu et al. 2004). A method that could analyze the contribution of each component trait to a complex trait, and the conditional effects and the conditional variance components for single developmental traits was proposed (Wen and Zhu 2005; Zhu 1995). This conditional model was combined with the QTL mapping method to effectively identify the influence of one trait on another or to study the developmental behavior of quantitative traits at the QTL level (Zhu 1999). To date, by analysis of conditional variance components and conditional genetic effects, conditional QTL analysis has been used for evaluating extra genetic variation and QTL effects of target traits conditioned on their components for grain yield (Guo et al. 2005; Liu et al. 2008) and panicle characteristics (Ye et al. 2009) of rice, popping expansion volume of maize (Li et al. 2008a), plant height of wheat (Cui et al. 2011), yield of cotton (Wu et al. 2004) and oil content of rapeseed (Zhao et al. 2006). Depending on the phenotype at various development stages, the method was also used to reveal the static genetic control of traits at different growth stages for plant height and tiller number of rice (Jiang et al. 2008), grain filling rate of maize (Liu et al. 2011), plant height (Wang et al. 2010; Wu et al. 2010; Zhang et al. 2011) and grain weight (Li et al. 2012) of wheat, and flower and pod numbers of soybean (Zhang et al. 2010). But there have been no studies dissecting QTLs based on trait values conditioned on different environments to study the influences of environmental factors on QTL expression.

The objectives of the present study were to map QTLs for 14 agronomic and yield traits and 8 N concentration, uptake and utilization efficiency traits in a recombinant inbred line (RIL) population under field condition across six different N or P supplement environments and identify molecular markers which may be useful in MAS breeding; to uncover the genetic effects of N or P fertilization by unconditional and conditional QTL analysis; and to discuss the availability of conditional analysis in dissecting QTLs induced by environmental factors.

Materials and methods

Plant materials

A population of 182 F_{11} RILs derived from a cross between wheat cultivars Xiaoyan 54 and Jing 411 was used in this study. Xiaoyan 54 was derived from Xiaoyan 6, a famous cultivar and founder parent of wheat that has been widely cultivated for the past 25 years in China. Xiaoyan 6 was derived from hybridization of wheat and *Thinopyrum ponticum* ($2n = 10x = 70$) and was characterized by high yield potential, wide environmental adaptability and good bread-making quality (Li et al. 2008b). Jing 411 was one of the main cultivars at the Northern Winter Wheat Region of China in the 1990s and had been widely grown on as much as 1.87 million ha (Zhuang 2003).

Experimental design

The experiments were conducted at Luancheng Agro-ecosystem Experimental Station, the Chinese Academy of Sciences (37°53′15″N, 114°40′47″E, and elevation 50 m, located at the piedmont of the Taihang Mountains in the North China Plain) during the 2006–2007 and 2007–2008 growing seasons. Three treatments were applied: low N (LN), low P (LP) and normal fertilized control (CK). Hereafter, '2006LN', '2006LP', '2006CK', '2007LN', '2007LP' and '2007CK' represent the six year × treatment trials, respectively.

A split-plot factorial (RIL × treatment) block design was employed, with three separate adjacent blocks as the main plots for the three treatments and subplots for the 182 RILs and their parents. In each plot, the RILs and their parents were arranged according to plant height obtained in pre-experiments. Two replications were grown in each plot. A 1.5 m^2 subplot with four 1.5 m-long rows, 0.25 m apart, and 30 seeds for each row were used. Seeds were hand planted at the beginning of October, and plants were harvested in the middle of next June at physiological maturity.

To analyze the soil N and P content, nine samples were selected for 0–20 cm depth before sowing using a diagonal sampling method for each block of both years. The values of soil N and P content were the average of the nine samples. The soil nitrate-N content in the 2006LN, 2006LP, 2006CK, 2007LN, 2007LP and 2007CK environments was 22.9, 29.5, 29.3, 23.3, 26.5 and 25.5 mg kg^{-1}, respectively, which was obtained as described by Wang et al. (2011), while the soil available P was 7.0, 4.5, 6.5, 7.1, 4.3 and 6.4 mg kg^{-1}, respectively. In the CK and LP plots, N was applied as urea at 120 kg N ha^{-1} before sowing and 60 kg N ha^{-1} at the stem elongation stage, whereas no N fertilizer was supplied to the LN plots. In the CK and LN plots, P was supplied as calcium superphosphate at 65 kg P ha^{-1} before sowing, with no P for LP plots. The fertilizer application rate of 180 kg N ha^{-1} and 65 kg P ha^{-1} was the basic recommendation level in the North China Plain (Wang et al. 2011). No K fertilizer was applied for all treatments, since soil tests indicated high contents of K at this site.

A pre-seeding irrigation supplying 60 mm water and three more irrigation (before winter, at the setting stage, and at the filling stage) with 100 mm water each was applied to all treatments. The rainfall for the 2006–2007 and 2007–2008 growing seasons were 110 and 170 mm, respectively. Plots were kept free from weeds, insects and diseases by appropriate measures.

Trait measurement

In each plot, 20–30 plants in the middle of the two internal rows were sampled to investigate grain yield per plant (GY) and aboveground dry matter per plant (DM), with a subsample of ten plants to investigate the following traits: plant height (PH) and spike number per plant (SNP) were determined from the mean of the ten plants; spike length (SL), kernel number per spike (KNS), kernel weight per spike (KWS), sterile spikelet number per spike (SSS), fertile spikelet number per spike (FSS) and total spikelet number per spike (TSS) were determined from the mean of the main spikes of the ten plants. Thousand kernel weight (TKW) was evaluated after harvest by weighing three samples of 500 kernels from each plot. Harvest index (HI) was calculated as GY/DM, spikelet compactness (SCN) as TSS/SL and straw yield (SY) as DM–GY.

The grains and straws were separately milled using a cyclone sample mill with 0.5 mm mesh. Grain N concentration (GNC) and straw N concentration (SNC) were determined using a micro-Kjeldahl method (Kjeltec 2200 auto distillation unit, FOSS Tecator AB, Sweden).

The following parameters were calculated:

1. Grain nitrogen uptake (GNUP) = GY × GNC,
2. Straw nitrogen uptake (SNUP) = SY × SNC,
3. Nitrogen uptake (NUP) = GNUP + SNUP,
4. Nitrogen harvest index (NHI) = GNUP/NUP,
5. Nitrogen utilization efficiency for grain yield ($NUtE_{GY}$) = GY/NUP,
6. Nitrogen utilization efficiency for aboveground dry matter ($NUtE_{DM}$) = DM/NUP.

Data analysis and QTL mapping

Analysis of variance (ANOVA) of the data was performed using SPSS 16.0 software (SPSS Inc, Chicago, USA). The broad-sense heritability (h_B^2) was calculated using a model where the six environments were regarded as six

replications and the genotype × environment interaction as the error term (Guo et al. 2012). The linkage map of the "Xiaoyan 54 × Jing 411" population was used for QTL analysis. The map included 555 markers distributed on 21 wheat chromosomes, comprising 523 simple sequenced repeats (SSRs), 18 expressed sequence tag-SSRs (EST-SSRs) and 14 *Glu* loci, as described in our previous studies (Xu et al. 2012a, b). Conditional analysis was performed to study the effects of N or P fertilization on QTL expression. The conditional phenotypic values ($y_{(CK|LN)}$ and $y_{(CK|LP)}$) are the net genetic variation of trait values in CK independent of that in LN or LP, which were evaluated using QGAStation 1.0 (http://ibi.zju.edu.cn/software/qga/). Conditional genetic analysis was conducted using "Conditional on Final Stage" for traits in each separate year. Both the observed and the conditional phenotypic values were used for QTL analysis, and the QTLs identified were defined as unconditional QTLs and conditional QTLs, respectively. Mixed linear composite interval mapping was done in the software QTLNetwork 2.1 to map QTLs (Yang et al. 2008). Composite interval analysis was undertaken using forward–backward stepwise, multiple linear regression with 1 cM walking speed, 2D genome scan, a probability into and out of the model of 0.05 and window size set at 10 cM. Significant thresholds for QTL detection were calculated with 1,000 permutations and a genome-wide error rate of 0.10 (suggestive) and 0.05 (significant).

Results

Phenotypic variation and correlations among traits

The results of ANOVA, the heritability (h_B^2) values and mean phenotypic performance for the investigated traits of the RILs and their parents across the six environments are shown in Table 1, with the detailed statistics based on single environment reported in supplementary Table S1. The mean values of two parents showed significant difference for 13 of the 22 traits. The phenotypic values for the traits exhibited broad and continuous variation among the 182 RILs and significant transgressive segregation for both the parents (Table 1), which might be attributed to the different background of the two parents and the polygenic inheritance of the traits. The coefficient of variation (CV) ranged from 7.9 to 26.6 % for 20 of the 22 traits, with the other two traits NHI (averaged 4.2 %) and SSS (56.6 %) showing extreme values (Table 1).

The variance for either genotype or environment effects on all the 22 investigated traits was significant at the $p \leq 0.001$ (Table 1). The LSD test showed that the mean values of the investigated traits were significantly different in many cases between the six environments (Supplementary Table S1). These results indicated that both the environments and genetic background were very important in explaining the overall phenotypic variation. The traits SNP, GY and DM showed significant reduction after low P-input across the 2 years (Supplementary Table S1), while SNP, KNS, GY, SY and DM were significantly decreased after low N-input in the year 2006, but showed little decrease in the year 2007. The N concentration traits (GNC and SNC) and N uptake traits (GNUP, SNUP and NUP) were significantly reduced in low N-input environments across the 2 years except for SNC in the year 2007, but showed complex response after low P-input. Both $NUtE_{GY}$ and $NUtE_{DM}$ were significantly improved (20.6–28.8 %) after low N-input across the 2 years, but showed no significant response in low P-input environments. These results indicated that low N-input can decrease N concentration and N uptake, and subsequently increase NUtE. The low P-input had no significant effect on the above N related traits, but decreased SNP, GY and DM (Supplementary Table S1).

The h_B^2 of the 22 investigated traits ranged from 10.6 % (GNUP) to 78.8 % (PH) (Table 1). The 14 agronomic and yield traits showed higher h_B^2 values (averaged 41.3 %) than the 8 N concentration, uptake and utilization efficiency traits (averaged 21.2 %). The h_B^2 values of four traits PH, SL, SCN and TKW were higher than or almost near 70.0 %, while another five traits (KNS, KWS, HI, GNC and $NUtE_{GY}$) were more than 40.0 %.

Correlation coefficients among the 22 traits of the RILs in each environment are summarized in supplementary Table S2. The correlation coefficients for 818 out of 1386 trait × environment by trait × environment (818/1,386 × 100 % = 59.0 %) were significant. PH, SL and SCN were significantly correlated with each other across all of the six environments. The yield production traits (DM, GY and SY) exhibited much higher correlation coefficients with SNP rather than KNS or TKW and showed positive association with the N uptake traits (GNUP, SNUP and NUP) in very high correlation coefficients across all of the six environments. TSS was positively correlated with FSS across six environments with high correlation coefficients, while positively correlated with SSS in three environments with low correlation coefficients. N concentration traits (GNC and SNC) were negatively correlated with NUtE traits ($NUtE_{GY}$ and $NUtE_{DM}$).

Unconditional QTL analysis

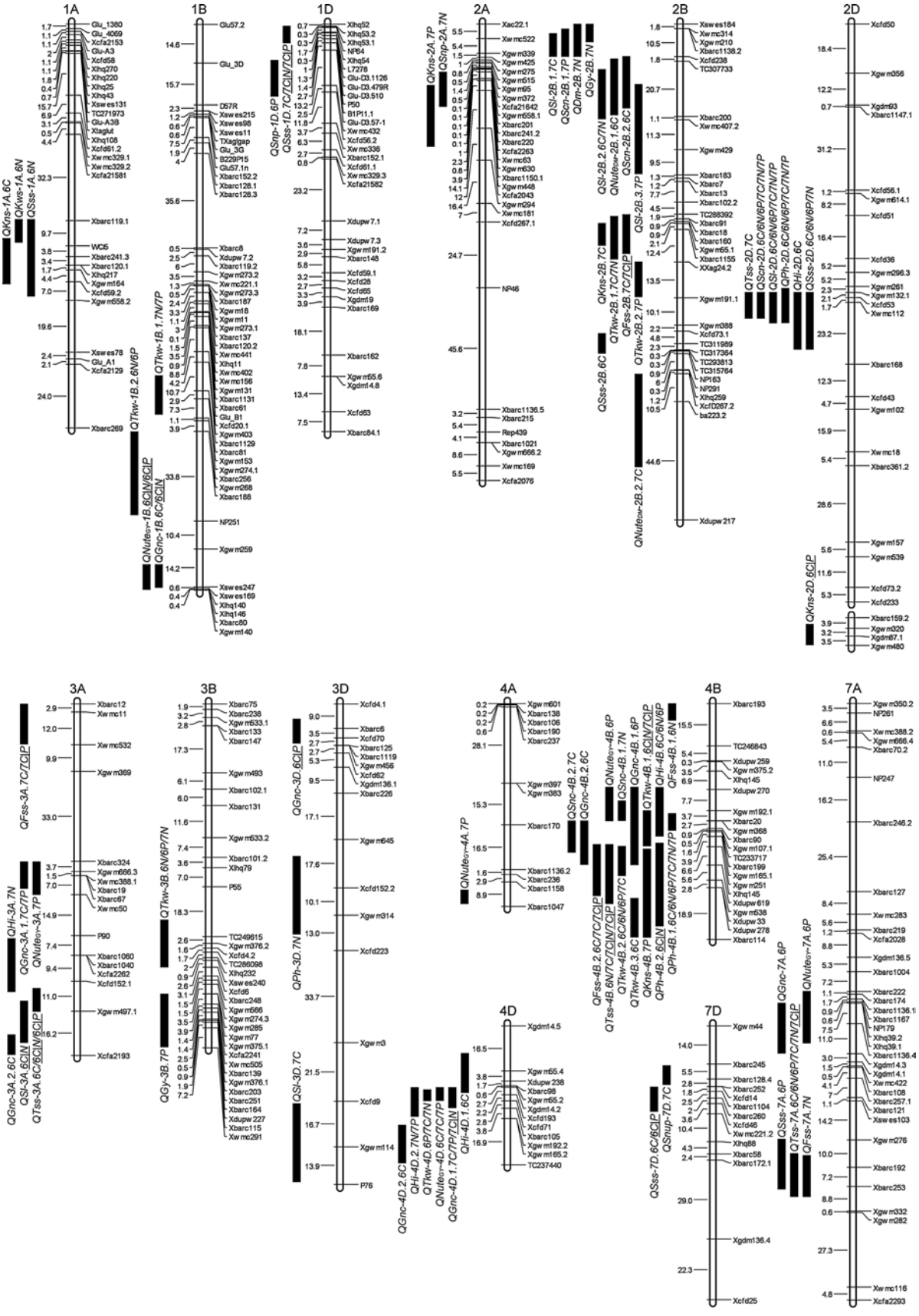
For the 22 traits studied, a total of 117 QTLs were detected (including 187 trait × environments), distributed on 20 of the 21 wheat chromosomes except for 7B (Supplementary Table S3; Fig. 1). Among them, 84 (141 trait × environments) were for the 14 agronomic and yield traits and

Table 1 Analysis of variance (ANOVA), the heritability (h_B^2) values and mean phenotypic performance for the investigated traits of the recombinant inbred lines (RILs) and their parents in six environments

Traits	Source of variation		h_B^2 (%)	Parents		RIL population				
	Genotypes	Environments		Xiaoyan 54	Jing 411	Average	Min.	Max.	SD	CV (%)
PH, plant height (cm)	23.3***	378.9***	78.8	73.3	84.8*	79.1	54.8	104.1	9.8	12.4
SL, spike length (cm)	20.1***	18.9***	76.1	7.1	8.0*	7.7	5.0	10.6	1.1	14.4
SCN, spikelet compactness (No. cm^{-1})	14.8***	36.3***	69.7	2.83	2.61*	2.56	1.80	3.69	0.36	13.9
SNP, spike number per plant (No. $plant^{-1}$)	2.1***	445.1***	15.4	5.82	5.87	5.74	3.63	9.00	0.98	17.1
KNS, kernel number per spike (No. $spike^{-1}$)	5.1***	53.8***	40.8	46.5	46.7	42.1	27.0	59.3	6.1	14.4
TKW, thousand kernel weight (g)	20.0***	212.5***	76.0	42.6	47.4*	44.7	32.2	55.9	4.3	9.6
KWS, kernel weight per spike (g $spike^{-1}$)	5.4***	65.1***	42.5	1.40	1.77*	1.43	0.94	2.02	0.21	14.7
TSS, total spikelet number per spike (No. $spike^{-1}$)	3.3***	51.5***	27.9	20	21	19	15	23	2	8.1
SSS, sterile spikelet number per spike (No. $spike^{-1}$)	2.3***	56.0***	17.7	2	2	2	0	5	1	56.6
FSS, fertile spikelet number per spike (No. $spike^{-1}$)	2.3***	14.7***	18.0	19	19	18	13	22	2	9.5
DM, aboveground dry matter (g $plant^{-1}$)	2.3***	339.2***	17.5	17.53	22.13*	18.47	10.80	28.01	3.41	18.9
GY, grain yield (g $plant^{-1}$)	2.9***	249.2***	23.7	7.93	10.36*	8.07	4.37	12.89	1.68	21.2
SY, straw yield (g $plant^{-1}$)	2.4***	374.4***	18.9	9.60	11.77*	10.40	5.74	16.42	2.03	20.1
HI, havest index	8.6***	139.8***	55.8	0.46	0.47	0.44	0.34	0.53	0.04	8.4
GNC, grain nitrogen concentration (%)	5.4***	535.3***	42.1	2.47	2.29*	2.55	1.94	3.24	0.25	9.9
SNC, straw nitrogen concentration (%)	2.4***	192.7***	18.5	0.43	0.41	0.46	0.27	0.71	0.08	16.9
GNUP, grain nitrogen uptake (g $plant^{-1}$)	1.7***	244.2***	10.6	0.193	0.234*	0.203	0.113	0.317	0.040	20.5
SNUP, straw nitrogen uptake (g $plant^{-1}$)	1.8***	332.7***	12.3	0.042	0.049*	0.049	0.023	0.083	0.013	26.6
NUP, nitrogen uptake (g $plant^{-1}$)	1.8***	280.0***	12.1	0.235	0.283*	0.252	0.136	0.396	0.050	20.4
NHI, nitrogen harvest index	2.5***	149.9***	20.4	0.824	0.831	0.810	0.689	0.890	0.034	4.2
$NUtE_{GY}$, N utilization efficiency for GY (g $g^{-1}N$)	5.3***	367.2***	41.7	33.6	36.5*	32.5	24.2	41.9	3.4	10.5
$NUtE_{DM}$, N utilization efficiency for DM (g $g^{-1}N$)	1.8***	604.1***	12.0	74.3	77.4	74.4	58.7	91.1	6.0	7.9

* The parents were significantly different at the 0.05 probability level

*** Significance at $p \leq 0.001$


1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
7A
7B
7D

◀ **Fig. 1** Locations of QTLs detected in six environments based on RILs derived from Xiaoyan 54 × Jing 411 by unconditional and conditional analysis. QTLs are indicated on the *left* side of each chromosome; markers are shown on the *right*. For QTLs detected in different environments, a *slash* is inserted to distinguish the environments. Conditional QTLs are *underlined*. The codes 6C, 6 N, 6P, 7C, 7 N and 7P represent unconditional QTLs detected in 2006CK, 2006LN, 2006LP, 2007CK, 2007LN and 2007LP environments; 6C|N, 6C|P, 7C|N and 7C|P represent conditional QTLs based on trait values of CK conditioned on LN or LP in the years 2006 and 2007, respectively

33 (46 trait × environments) for the 8 N concentration, uptake and utilization efficiency traits. The QTLs explained 1.6–35.2 % of the phenotypic variation, respectively (Supplementary Table S3). Thirty-four QTLs (including 104 trait × environments) were found in at least two environments, with the additive effects consistent across all the significant environments for each QTL. Twenty-four QTLs (20.5 % of the 117 QTLs) were effective in both years; among them 18 (15.4 % of the 117 QTLs, including 27 pairs of trait × treatments) were within at least one of the three nutritional treatments, with 14, 7 and 6 for CK, LN and LP treatments, respectively (Supplementary Table S3). Four QTLs were conserved across all of the six environments, and another 14 effective in three to five environments (Table 2). As to the six environments, 37, 31, 28, 41, 27 and 23 QTLs were found in 2006CK, 2006LN, 2006LP, 2007CK, 2007LN and 2007LP, respectively.

For PH, five QTLs were detected, three of which were found to be specific for a single environment (Supplementary Table S3; Fig. 1). Two major QTLs (*QPh-2D* and *QPh-4B*) showed significant effects across all of the six environments, and with contributions as high as 20.1 and 27.4 %, respectively (Table 2). The Xiaoyan 54-derived alleles decreased PH at four of the five QTLs except that on chromosome 3D.

Nine QTLs were identified for each of SL and SCN. Both parents contributed the favorable alleles at the QTLs of the two traits. Extensive overlap was observed between the QTLs for SL and SCN. In summary, seven common QTLs were found for the two traits, but all with opposite additive effects, which was consistent with the results of correlation analysis. The interval *Xcfd53–Xwmc112* on chromosome 2D was identical to *QPh-2D*, and could affect SL and SCN across all of the six environments.

For SNP, KNS and TKW, 3, 6 and 13 QTLs were detected, respectively. Both parents contributed favorable alleles for the QTLs of the three traits. Seven QTLs of TKW showed significant effects in at least two environments. The major QTL *QTkw-4B.1* was effective in four environments and explained 8.5–13.9 % of the phenotypic

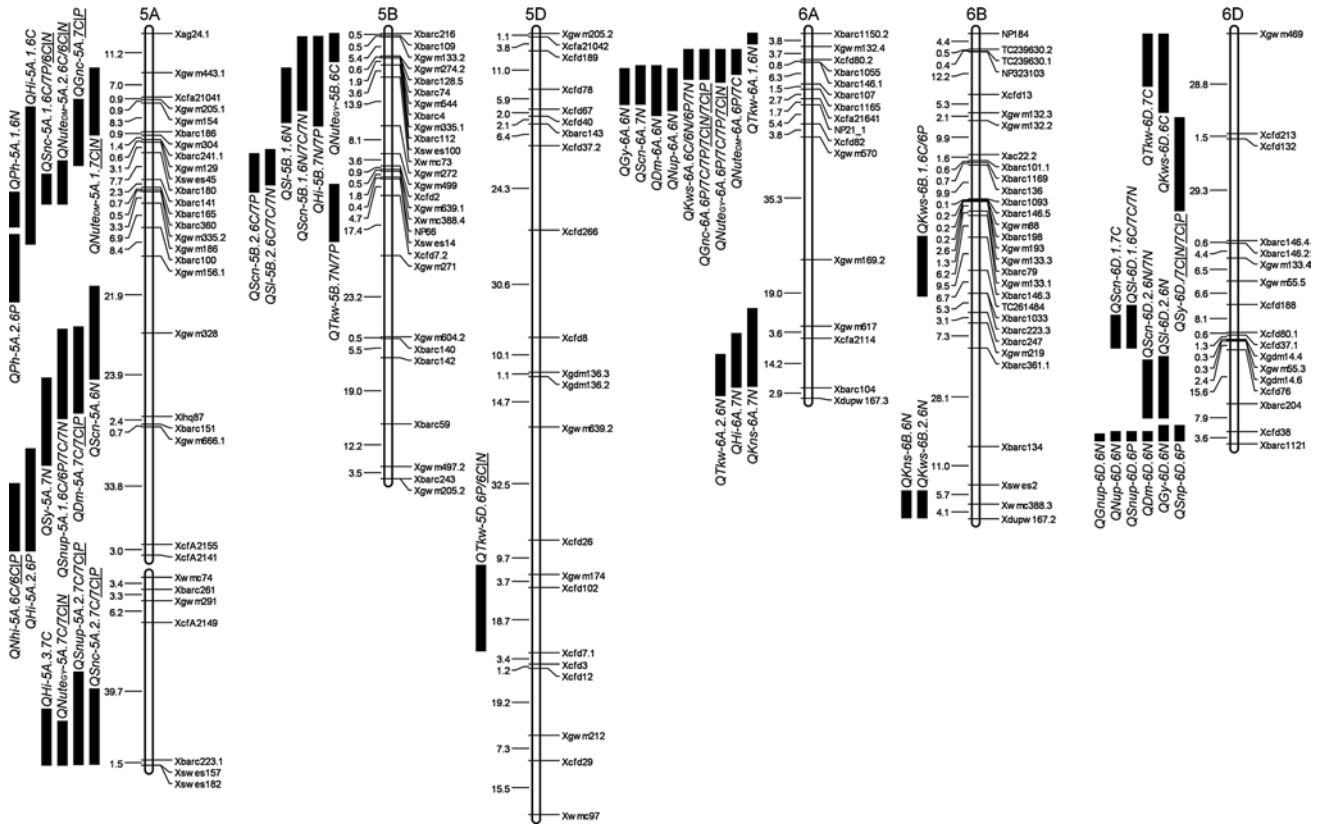


Fig. 1 continued

Table 2 Summary of unconditional QTLs detected in at least three environments

Traits[a]	QTLs	Marker intervals[b]	2006CK[c]	2006LN	2006LP	2007CK	2007LN	2007LP
PH	*QPh-2D*	*Xcfd53–Xwmc112*	−5.038	−4.249	−4.537	−4.763	−4.423	−3.894
			18.0	17.1	21.5	23.5	20.9	19.9
	QPh-4B	*Xbarc20–Xbarc90*	−6.210	−6.040	−5.620	−5.303	−5.426	−4.238
			30.8	26.9	26.3	27.7	30.6	21.9
SL	*QSl-2D*	*Xcfd53–Xwmc112*	−0.613	−0.545	−0.660	−0.637	−0.657	−0.676
			28.4	30.4	35.2	31.0	31.6	30.4
	QSl-5B.2	*Xgwm272–Xswes14*	0.354			0.305	0.299	
			4.6			2.3	3.0	
	QSl-6D.1	*Xcfd80.1–Xcfd37.1–Xgdm14.4*	−0.308			−0.303	−0.270	
			8.3			7.5	5.7	
TKW	*QTkw-3B*	*TC249615–Xgwm376.2*	−0.970		−1.010	−0.877		
			7.8		8.0	5.7		
	QTkw-4B.1	*Xlhq145–Xdupw619*	−1.157	−1.237	−1.793	−1.886		
			13.9	8.5	11.6	13.3		
	QTkw-4D	*Xcfd193–Xcfd71*			1.343	0.895	1.456	
					13.2	4.7	7.5	
KWS	*QKws-6A*	*Xcfd80.2–Xbarc1055*	−0.056	−0.081	−0.067	−0.064		
			9.3	18.2	12.2	8.0		
TSS	*QTss-7A*	*Xbarc192–Xbarc253*	−0.461	−0.707	−0.550	−0.798	−0.806	
			7.1	15.5	12.3	20.5	18.0	
SSS	*QSss-2D*	*Xwmc112–Xbarc168*	−0.283	−0.386	−0.311	−0.281		
			10.5	11.4	10.6	8.0		
HI	*QHi-4B*	*Xgwm192.1–Xbarc20*	0.012	0.014	0.016			
			12.2	13.7	16.7			
SCN	*QScn-2D*	*Xcfd53–Xwmc112*	0.196	0.195	0.229	0.152	0.181	0.161
			26.0	29.9	32.8	22.2	27.4	22.6
	QScn-5B.1	*Xgwm133.2–Xbarc112–Xwmc73*		−0.148		−0.100	−0.121	
				5.5		7.0	6.2	
GNC	*QGnc-6A*	*Xcfd80.2–Xbarc1055*			0.081	0.131		0.084
					9.4	15.4		8.9
SNUP	*QSnup-5A.1*	*Xgwm328–Xlhq87*	0.008		0.006	0.004	0.004	
			8.2		9.1	7.1	8.9	
$NUtE_{GY}$	$QNUtE_{GY}$*-4D*	*Xgdm14.2–Xcfd193–Xcfd71*	−0.765			−0.966		−0.989
			8.8			9.1		10.7
	$QNUtE_{GY}$*-6A*	*Xcfd80.2–Xbarc1055*			−0.988	−1.336		−0.787
					9.7	12.3		7.2

[a] The abbreviations of traits can refer to Table 1

[b] Marker interval means the interval of the *F*-value peak for QTLs

[c] The QTLs were all significant at the 0.001 probability level. The above and below values indicated the additive effects and phenotypic variations explained by the QTLs. Positive effect, increased effect contributed by Xiaoyan 54; negative effect was contributed by Jing 411

variation. The interval *Xbarc1150.1–Xgwm448–Xcfa2043* on chromosome 2A was detected to affect SNP and KNS simultaneously, while three intervals on chromosomes 2B, 4B and 6A were found for KNS and TKW, but all with opposite additive effects.

Five QTLs were detected for KWS, with Jing 411 contributing the favorable alleles for all of them. The major QTL *QKws-6A* was effective in four environments and explained 8.0–18.2 % of the phenotypic variation, while another QTL *QKws-6B.1* could affect KWS in two environments.

Four, six and five QTLs were identified for TSS, SSS and FSS, respectively. Both Xiaoyan 54- and Jing 411-derived alleles contributed to the additive effects of the

three traits. TSS and SSS were co-located on chromosome 2D, with Jing 411-derived allele having increased TSS in 2007CK and SSS in four environments. TSS and FSS were co-located on chromosome 4B, with Xiaoyan 54-derived allele having the two traits increased in two environments, respectively. The interval *Xgwm276–Xbarc192–Xbarc253* on chromosome 7A affected all the three traits, with contributions ranging between 7.1 and 20.5 % for TSS across five environments.

Four QTLs were identified for each of DM and GY, and one for SY. DM and GY were co-located on chromosomes 2B, 6A and 6D, while DM and SY were co-located on chromosome 5A. Xiaoyan 54-derived alleles had positive effects on chromosomes 2B and 5A, whereas Jing 411-derived allele increased the traits on chromosomes 6A and 6D.

For HI, a total of ten QTLs were detected, three of which were identified in at least two environments. Xiaoyan 54 and Jing 411 contributed positive alleles to five QTLs each. The locus *QHi-4B* was effective across the three environments in the year 2006, while *QHi-4D.2* and *QHi-5B* showed significant effects in 2007LN and 2007LP.

Nine, four, eight and four QTLs were identified for GNC, SNC, $NUtE_{GY}$ and $NUtE_{DM}$, respectively. The four QTLs for SNC were contributed by Xiaoyan 54-derived alleles, while both parents contributed the favorable alleles for the other three traits. Seven overlap intervals for the N concentration and NUtE traits were found on chromosomes 3A, 4B, 4D, 5A, 6A and 7A, but all with opposite additive effects, showing consistency with their negative correlations. The intervals on chromosomes 3A, 4D, 5A and 6A were detected in at least two environments.

One, four and two QTLs were detected for GNUP, SUNP and NUP, respectively. The QTLs for GNUP and NUP were all contributed by Jing 411-derived alleles, whereas those for SNUP came from Xiaoyan 54-derived alleles. The QTL *QSnup-5A.1* showed significant effects in four environments. The interval *Xcfd38–Xbarc1121* on chromosome 6D affected all of the three traits.

Only one QTL, *QNhi-5A*, conferred by Jing 411-derived allele, was detected for NHI. It was co-located with *QHi-5A.2*.

Conditional QTL analysis

A total of 30 conditional QTLs were detected in 22 chromosome intervals (Supplementary Table S3; Fig. 1). Among them, 21 were also identified in unconditional analysis; while 9 were newly detected QTLs. Fifteen QTLs were found for either morphological or physiological traits. The QTLs explained 4.9–18.6 % of the phenotypic variation, respectively. Seven QTLs were identified conditioned either on LN or LP environments, with six in 1 year and the other one in different years.

By comparing the QTL effects of unconditional analysis and conditional analysis based on trait values of CK conditioned on that of LN or LP, we can evaluate the effects of N or P fertilization on QTL expression of related traits. For example, if a conditional QTL conditioned on LN has a similar or greatly different effect to its unconditional QTL, it demonstrates that the QTL is completely or partially contributed by the N supplement. Whereas if an unconditional QTL is unable to be detected again when conditioned on LN, the QTL is considered to be not controlled by N supplement.

Conditional analysis detected three, two and three of the four, six and five unconditional QTLs for TSS, SSS and FSS, respectively (Table 3; Fig. 1). The QTLs *QTss-3A* and *QSss-1D* were detected by unconditional analysis in CK and by conditional analysis when conditioned on LN or LP with similar contributions, indicating that QTLs could express only when both N and P fertilizers were introduced. The QTLs *QTss-7A*, *QSss-7D*, *QFss-2B*, *QFss-3A* and *QFss-4B.2* were identified in CK and when conditioned on LP environments. They may be P-contributed QTLs. The QTL *QTss-4B* was detected in 2006LN and 2007CK and when conditioned on LN and LP in 2007. In the same interval, P fertilization induced the expression of *QFss-4B.2*. Considering the contributions of the QTLs, this locus may be induced by P fertilization and partially by N fertilization; N and P may have interactive relationship on the expression of *QTss-4B*.

Four, two, two and one of the nine, four, eight and four unconditional QTLs for GNC, SNC, $NUtE_{GY}$ and $NUtE_{DM}$ were detected by conditional analysis, respectively (Table 3; Fig. 1). Additional two, one and one conditional QTLs that failed to be found in unconditional analysis were identified for GNC, $NUtE_{GY}$ and $NUtE_{DM}$, respectively. The QTLs *QGnc-1B*, *QGnc-3A.2*, *QGnc-4D.1*, *QSnc-5A.1*, *QNUtE$_{GY}$-5A*, *QNUtE$_{GY}$-6A* and *QNUtE$_{DM}$-5A.2* were detected by unconditional analysis in CK and by conditional analysis when conditioned on LN with similar contributions, while *QNUtE$_{DM}$-5A.2* was found only when conditioned on LN. These QTLs may be N fertilization-induced QTLs. The QTLs *QGnc-3D*, *QGnc-5A* and *QSnc-5A.2* were detected by conditional analysis when conditioned on LP, indicating to be P-induced QTLs. The QTL *QGnc-6A* was detected by unconditional analysis in CK and LP environments and by conditional analysis when conditioned on LN and LP environments. By comparing the contributions of the QTLs, we found N induced the expression of the QTL, while P facilitated the effects of N.

Furthermore, one new conditional QTL was detected for each of PH (N induced), SL (N induced), KNS (P induced), TKW (N and P induced) and SY (P induced)

Table 3 Conditional QTLs of traits for spike number per spike (TSS, SSS and FSS), nitrogen concentration (GNC and SNC) and utilization efficiency ($NUtE_{GY}$ and $NUtE_{DM}$)

QTLs	2006					2007				
	CK	LN	LP	CK|LN	CK|LP	CK	LN	LP	CK|LN	CK|LP
QTss-3A	0.514			0.704	0.497					
	9.3			11.2	9.0					
QTss-4B		0.471				0.488			0.411	0.497
		7.9				12.5			9.5	11.2
QTss-7A	−0.461	−0.707	−0.550			−0.798	−0.806			−0.795
	7.1	15.5	12.3			20.5	18.0			18.6
QSss-1D						0.306			0.271	0.287
						9.0			8.1	8.2
QSss-7D	0.195				0.197					
	6.8				8.1					
QFss-2B						0.494				0.459
						10.7				9.3
QFss-3A						−0.429				−0.439
						8.5				9.0
QFss-4B.2	0.527					0.488				0.483
	8.2					10.1				9.7
QGnc-1B	0.048			0.053						
	8.6			10.2						
QGnc-3A.2	0.061			0.062						
	7.5			8.2						
QGnc-3D					0.047					
					8.4					
QGnc-4D.1						0.115		0.096	0.090	
						10.7		9.5	6.5	
QGnc-5A										−0.085
										9.9
QGnc-6A			0.081			0.131		0.084	0.121	0.067
			9.4			15.4		8.9	14.7	5.8
QSnc-5A.1	0.030			0.032				0.022		
	11.9			8.9				12.0		
QSnc-5A.2						0.028				0.033
						7.1				9.4
$QNUtE_{GY}$-*1B*				−0.820	−0.638					
				10.7	8.6					
$QNUtE_{GY}$-*5A*						−1.090			−1.066	
						9.0			8.3	
$QNUtE_{GY}$-*6A*			−0.988			−1.336		−0.787	−1.101	
			9.7			12.3		7.2	9.0	
$QNUtE_{DM}$-*5A.1*									1.147	
									4.9	
$QNUtE_{DM}$-*5A.2*	−1.462			−1.495						
	9.7			9.3						
$QNUtE_{DM}$-*6A*			−1.387			−1.717				
			9.2			12.2				

(Supplementary Table S3; Fig. 1), while one unconditional QTL was also identified in conditional analysis for each of TKW (N induced), DM (P induced), SNUP (P induced) and NHI (P induced) (Supplementary Table S3; Fig. 1).

Important QTL clusters

A total of 34 QTL clusters were identified, among which 6 were with one trait in at least two environments and 28 were related to more than one trait (Table 4; Fig. 1). Five intervals harboring various QTLs were of utmost importance.

The interval *Xcfd53–Xwmc112* on chromosome 2D affected PH, SL and SCN across all the six environments. The Jing 411-derived allele increased PH and SL, but decreased SCN, with relatively high contributions (Tables 2 and 4). Besides, the Jing 411-derived allele also increased SSS in four environments and TSS in 2007CK, and decreased HI in 2006CK. *Xbarc20–Xbarc90* on chromosome 4B was another interval which affected PH greatly across the six environments, with Xiaoyan 54 conferring the favorite allele. The two intervals may be alleles of *Rht8* (Korzun et al. 1998; Worland et al. 1998; Worland et al.

Table 4 Summary of QTL clusters with at least two trait × environment

Chr	Intervals[a]	No.	Traits (additive effect[b], number of environments)
1A	*Xbarc119.1–WCI5*	2	KWS (−, 1), SSS (+, 1)
1B	*Xbarc81–Xgwm153*	2	TKW (−, 2)
	Xbarc188–NP251	2	TKW (−, 2)
2A	*Xbarc1150.1–Xgwm448–Xcfa2043*	2	SNP (−, 1), KNS (+, 1)
2B	*Xswes184–Xwmc314–Xcfd238*	4	GY (+, 1), DM (+, 1), SCN (−, 1), SL (+, 1)
	TC307733–Xbarc200	4	SL (+, 2), SCN (−, 1), $NUtE_{DM}$ (+, 1)
	Xbarc160–Xbarc1155–Xxag24.2	4	KNS (+, 1), TKW (−, 2), FSS (+, 1)
2D	*Xgwm132.1–Xcfd53–Xwmc112–Xbarc168*	24	PH (−, 6), SL (−, 6), SCN (+, 6), TSS (−, 1), SSS (−, 4), HI (+, 1)
3A	*Xgwm666.3–Xbarc19–Xwmc50*	3	GNC (−, 2), $NUtE_{GY}$ (+, 1)
3B	*TC249615–Xgwm376.2*	3	TKW (−, 3)
4B	*Xdupw270–Xgwm192.1–Xbarc20*	6	GNC (−, 1), $NUtE_{GY}$ (+, 1), SNC (+, 1), HI (+, 3)
	Xbarc20–Xbarc90	6	PH (−, 6)
	Xbarc90–Xgwm107.1–Xgwm251	2	SNC (+, 1), GNC(−, 1)
	Xlhq145–Xdupw619–Xgwm538–Xdupw33	8	TKW (−, 4), FSS (+, 2), TSS (+, 2)
	Xdupw278–Xbarc114	2	KNS (+, 1), TKW (−, 1)
4D	*Xgdm14.2–Xcfd193–Xcfd71*	10	GNC (+, 2), $NUtE_{GY}$ (−, 3), TKW (+, 3), HI (−, 2)
5A	*Xbarc165–Xbarc360–Xgwm335.2*	4	$NUtE_{DM}$ (−, 1), SNC (+, 2), HI (+, 1)
	Xgwm328–Xlhq87	7	SNUP (+, 4), DM (+, 1), SCN (+, 1), SY (+, 1)
	Xgwm666.1–Xcfa2155	2	HI (−, 1), NHI (−, 1)
	Xcfa2149–Xbarc223.1	4	SNC (+, 1), SNUP (+, 1), $NUtE_{GY}$ (−, 1), HI (−, 1)
5B	*Xgwm133.2–Xbarc112–Xwmc73*	6	HI (−, 2), SCN (−, 3), SL (+, 1)
	Xgwm272–Xswes14–Xcfd7.2	5	SCN (−, 2), SL (+, 3)
	Xcfd7.2–Xgwm271	2	TKW (+, 2)
6A	*Xcfd80.2–Xbarc1055*	12	$NUtE_{DM}$ (−, 2), $NUtE_{GY}$ (−, 3), GNC (+, 3) KWS (−, 4)
	Xbarc107–Xbarc1165–Xcfa21641	4	NUP (−, 1), DM (−, 1), GY (−, 1), SCN (+, 1)
	Xcfa2114–Xbarc104	3	KNS (+, 1), HI (+, 1), TKW (−, 1)
6B	*Xbarc146.3–TC261484*	2	KWS (−, 2)
	Xwmc388.3–Xdupw167.2	2	KNS (−, 1), KWS (−, 1)
6D	*Xgwm469–Xcfd213*	2	TKW (−, 1), KWS (−, 1)
	Xcfd80.1–Xgdm14.4	4	SL (−, 3), SCN (+, 1)
	Xcfd76–Xbarc204	3	SL (−, 1), SCN (+, 2)
	Xcfd38–Xbarc1121	6	SNUP (+, 1), GNUP (−, 1), NUP (−, 1), SNP (+, 1), DM (−, 1), GY (−, 1)
7A	*Xlhq39.1–Xbarc1136.4–Xgdm14.3*	2	$NUtE_{GY}$ (−, 1), GNC (+, 1)
	Xgwm276–Xbarc192–Xbarc253	7	TSS (−, 5), FSS (−, 1), SSS (−, 1)

[a] Marker interval means the interval of the *F*-value peak for QTLs

[b] Positive effect, increased effect contributed by Xiaoyan 54; negative effect was contributed by Jing 411

2001) and *Rht-B1b* (Börner et al. 1996; Liu et al. 2012) genes, respectively.

The interval *Xlhq145–Xdupw619* on chromosome 4B showed significant effects for TKW across four environments and for FSS and TSS in two environments. The Jing 411-derived allele increased TKW with relatively high contributions, but decreased FSS and TSS.

The interval *Xcfd80.2–Xbarc1055* on chromosome 6A affected KWS in four environments, with Jing 411 contributing the favorable allele. Besides, the Xiaoyan 54-derived allele in the interval increased GNC in three environments, but decreased $NUtE_{DM}$ and $NUtE_{GY}$ in two and three environments, respectively.

The interval *Xbarc192–Xbarc253* on chromosome 7A affects TSS across five environments except 2007LP, SSS in 2006LP and FSS in 2007LN simultaneously. The parent Jing 411 contributed the favorable allele in the interval.

Discussion

The effects of N and P deficiency on yield production and NUtE

By evaluating traits in different environments or treatments, we can obtain the influence of target environmental factors on QTL expression. Trials at different levels of N or P have been conducted to determine whether the expression of a QTL is constitutive, and to identify nutrient stress-specific and constitutively expressed traits in wheat (An et al. 2006; Guo et al. 2012; Laperche et al. 2007; Li et al. 2007b; Su et al. 2006, 2009). In the present study to dissect QTLs in different N or P supplement environments across 2 years, 11 QTLs were detected in all of the LN, LP and CK environments, among which 4 were identified across the six environments, indicating they were not (or not totally) influenced by N or P fertilization (Supplementary Table S3; Fig. 1). Twenty-eight and 19 QTLs were identified only in LN or LP environments, respectively. These may be low N- or low P-induced QTLs. For GNC, SNC, $NUtE_{GY}$ and $NUtE_{DM}$, a total of 25 QTLs located in 15 intervals were detected, but only 1 (*QSnc-4B.1*) was identified for SNC in low N-input environment (Supplementary Table S3; Fig. 1), which indicated the importance of N supplement on the QTL expression of the four N concentration and NUtE traits.

Zhu (1999) combined the conditional genetic analysis approach (Zhu 1995) with QTL mapping to identify the influence of one trait on another and to study the developmental behavior of quantitative traits at the individual QTL level. The present study applied this method to dissect QTLs based on trait values conditioned on different N or P supplement environments to study the genetic contribution of N and P fertilization on QTL expression of yield production and NUtE related traits. The traits for spikelet number per spike (eight QTLs), N concentration (eight QTLs) and NUtE (five QTLs) occupied 21 of the 30 conditional QTLs, indicating strong relationships of the traits with N or P fertilization (Table 3 and Supplementary Table S3). By comparing the unconditional and conditional QTLs, we found that P fertilization induced QTLs for spikelet number per spike mostly, whereas N fertilization had more effects on the expression of QTLs for N concentration and NUtE traits. The reason may be that for given cultivars, N supplement may increase root absorption of N element, resulting in increased N concentration, but decreased NUtE due to the law of diminishing marginal returns (Wang et al. 2011). Furthermore, N and P interactive relationship was detected in the expression of *QTkw-4B.1*, *QTss-3A*, *QTss-4B*, *QSss-1D* and *QGnc-6A* (Supplementary Table S3).

Important clusters and QTL comparison

QTL clustering was reported in many previous studies (Groos et al. 2003; Guo et al. 2012; Li et al. 2007a; Marza et al. 2006; Quarrie et al. 2005; Sun et al. 2009; Xu et al. 2012a). In the present study, a total of 34 QTL clusters were identified, among which 5 were more important (Table 4; Fig. 1).

The intervals *Xcfd53–Xwmc112* on chromosome 2D and *Xbarc20–Xbarc90* on chromosome 4B were identical to genes *Rht8* (Korzun et al. 1998; Worland et al. 1998; Worland et al. 2001) and *Rht-B1b* (Börner et al. 1996; Liu et al. 2012), respectively. In one of our previous studies using "Xiaoyan 54 × Jing 411" RIL population, the intervals were identified to affect shoot height and biomass production at the seedling stage in both salt stress and control treatments (Xu et al. 2012a). In this study, they were also detected to have pleiotropic effects for PH, SL, HI and N concentration and NUtE traits. The Xiaoyan 54 derived alleles decreased PH, but increased biomass production and HI at both intervals. One subsequent study to determine the transmit patterns of the important genomic regions of the founder parent Xiaoyan 6 (the variety from which Xiaoyan 54 was derived) to its derivative varieties indicated that the two intervals were strongly selected in wheat breeding practice. They were transmitted to the first, second and third generations of derivatives at proportions of 40.0, 37.5 and 45.5 %, and 73.3, 58.3 and 54.5 %, respectively (unpublished data). The proportions were relatively high considering their multi-allele nature, and did not show a sharp decline following the continuity of generations as expected. The results indicated that Xiaoyan 6 alleles at the two intervals (the same as Xiaoyan 54 alleles) have positive effects for wheat breeding and gave us a reasonable explanation for the formation of the founder parent Xiaoyan 6.

Considering that the two intervals were identified at both seedling and adult stages, and across various stress and normal environments, we can define them as non-environment-specific or non-stage-specific genes (Liu et al. 2008).

The interval *Xcfd80.2–Xbarc1055* on chromosome 6A contained QTLs for KWS, GNC, $NUtE_{DM}$ and $NUtE_{GY}$, all detected in at least two environments. This interval was identical to the gene *TaGW2*, which was associated with grain weight in bread wheat (Su et al. 2011). The other two intervals, *Xlhq145–Xdupw619* on chromosome 4B and *Xbarc192–Xbarc253* on chromosome 7A showed significant effects for yield component traits in various environments and with high contributions. These non-environment-specific QTLs may express stably in different environments and of great value for MAS breeding in wheat.

Besides, there were many other interesting clusters. For example, seven intervals for SL and SCN on chromosomes 2B (2), 2D, 5B (2) and 6D (2), three intervals for KNS and TKW on chromosomes 2B, 4B and 6A, and seven intervals for N concentration and NUtE on chromosomes 2D, 4B, 4D, 5A (2), 6A and 7A, which contained QTLs for both traits, but with negative additive effects. The results were in correspondence with the negative correlations between the related traits.

Conclusion

The present study provided an opportunity for the detection of QTLs induced by N and/or P fertilization and those with consistent effects across LN, LP and normal fertilization environments, and gave a direct and strong evidence for the availability of conditional analysis based on traits evaluated in different environments to dissect QTLs induced by environmental factors. The two major QTLs on chromosomes 2D and 4B for PH and the one on chromosome 6A for KWS, GNC and NUtE traits confirmed their importance as described by previous studies, while those for TKW on chromosome 4B and TSS on chromosome 7A have not already been reported in wheat. These QTL intervals, especially the newly detected ones, warrant further study to fine map, clone and elucidate their functional basis, and their positive alleles should be selected actively in wheat breeding. The detection of QTLs that interact with N and/or P supplements may provide insights into understanding the mechanism of wheat adaptation to nutrient-deficient environmental conditions.

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References

An DG, Su JY, Liu QY, Zhu YG, Tong YP, Li JM, Jing RL, Li B, Li ZS (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). Plant Soil 284:73–84

Börner A, Plaschke J, Korzun V, Worland AJ (1996) The relationships between the dwarfing genes of wheat and rye. Euphytica 89:69–75

Clárk RB (1983) Plant genotype differences in the uptake, translocation, accumulation, and use of mineral elements required for plant growth. Plant Soil 72:175–196

Cui F, Li J, Ding AM, Zhao CH, Wang L, Wang XQ, Li SS, Bao YG, Li XF, Feng DS, Kong LR, Wang HG (2011) Conditional QTL mapping for plant height with respect to the length of the spike and internode in two mapping populations of wheat. Theor Appl Genet 122:1517–1536

Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3:43–52

FAO (2003) *Food Outlook. No. 4*. FAO global information and early warning system on food and agriculture. FAO, Rome

Fontaine J-X, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. Theor Appl Genet 119:645–662

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–892

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032–1040

Guo LB, Xing YZ, Mei HW, Xu CG, Shi CH, Wu P, Luo LJ (2005) Dissection of component QTL expression in yield formation in rice. Plant Breeding 124:127–132

Guo Y, Kong FM, Xu YF, Zhao Y, Liang X, Wang YY, An DG, Li SS (2012) QTL mapping for seedling traits in wheat grown under varying concentrations of N, P and K nutrients. Theor Appl Genet 124:851–865

Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. Theor Appl Genet 114:403–419

Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369–2387

Jiang H, Jiang L, Guo LB, Gao ZY, Zeng DL, Zhu LH, Liang GH, Qian Q (2008) Conditional and unconditional mapping of quantitative trait loci underlying plant height and tiller number in rice (*Oryza sativa* L.) grown at two nitrogen levels. Prog Nat Sci 18:1539–1547

Korzun V, Röder M, Ganal M, Worland A, Law C (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 96:1104–1109

Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B (2006) A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. Theor Appl Genet 113:1131–1146

Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype × nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. Theor Appl Genet 115:399–415

Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. Eur J Agron 12:163–173

Li SS, Jia JZ, Wei XY, Zhang XC, Li LZ, Chen HM, Fan YD, Sun HY, Zhao XH, Lei TD, Xu YF, Jiang FS, Wang HG, Li LH (2007a) A intervarietal genetic map and QTL analysis for yield traits in wheat. Mol Breeding 20:167–178

Li ZX, Ni ZF, Peng HR, Liu ZY, Nie XL, Xu SB, Liu G, Sun QX (2007b) Molecular mapping of QTLs for root response to phosphorus deficiency at seedling stage in wheat (*Triticum aestivum* L.). Prog Nat Sci 17:1177–1184

Li YL, Dong YB, Cui DQ, Wang YZ, Liu YY, Wei MG, Li XH (2008a) The genetic relationship between popping expansion volume and two yield components in popcorn using unconditional and conditional QTL analysis. Euphytica 162:345–351

Li ZS, Li B, Tong YP (2008b) The contribution of distant hybridization with decaploid *Agropyron elongatum* to wheat improvement in China. J Genet Genomics 35:451–456

Li SP, Wang CS, Chang XP, Jing RL (2012) Genetic dissection of developmental behavior of grain weight in wheat under diverse temperature and water regimes. Genetica 140:393–405

Liu GF, Yang J, Xu HM, Hayat Y, Zhu J (2008) Genetic analysis of grain yield conditioned on its component traits in rice (*Oryza sativa* L.). Aust J Agr Res 59:189–195

Liu ZH, Ji HQ, Cui ZT, Wu X, Duan LJ, Feng XX, Tang JH (2011) QTL detected for grain-filling rate in maize using a RIL population. Mol Breeding 27:25–36

Liu YN, He ZH, Appels R, Xia XC (2012) Functional markers in wheat: current status and future prospects. Theor Appl Genet 125:1–10

Marza F, Bai GH, Carver BF, Zhou WC (2006) Quantitative trait loci for yield and related traits in the wheat population Ning7840 × Clark. Theor Appl Genet 112:688–698

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Rengel Z, Marschner P (2005) Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytol 168:305–312

Saal B, von Korff M, Leon J, Pillen K (2011) Advanced-backcross QTL analysis in spring barley: IV. Localization of QTL × nitrogen interaction effects for yield-related traits. Euphytica 177:223–239

Su JY, Xiao YM, Li M, Liu QY, Li B, Tong YP, Jia JZ, Li ZS (2006) Mapping QTLs for phosphorus-deficiency tolerance at wheat seedling stage. Plant Soil 281:25–36

Su JY, Zheng Q, Li HW, Li B, Jing RL, Tong YP, Li ZS (2009) Detection of QTLs for phosphorus use efficiency in relation to agronomic performance of wheat grown under phosphorus sufficient and limited conditions. Plant Sci 176:824–836

Su ZQ, Hao CY, Wang LF, Dong YC, Zhang XY (2011) Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 122:211–223

Sun XY, Wu K, Zhao Y, Kong FM, Han GZ, Jiang HM, Huang XJ, Li RJ, Wang HG, Li SS (2009) QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica 165:615–624

Wang ZH, Wu XS, Ren Q, Chang XP, Li RZ, Jing RL (2010) QTL mapping for developmental behavior of plant height in wheat (*Triticum aestivum* L.). Euphytica 174:447–458

Wang RF, An DG, Hu CS, Li LH, Zhang YM, Jia YG, Tong YP (2011) Relationship between nitrogen uptake and use efficiency of winter wheat grown in the North China Plain. Crop Pasture Sci 62:504–514

Wen YX, Zhu J (2005) Multivariable conditional analysis for complex trait and its components. Acta Genet Sin 32:289–296

Worland A, Korzun V, Röder M, Ganal M, Law C (1998) Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor Appl Genet 96:1110–1120

Worland A, Sayers E, Korzun V (2001) Allelic variation at the dwarfing gene *Rht8* locus and its significance in international breeding programmes. Euphytica 119:157–161

Wu JX, Jenkins JN, McCarty JC, Zhu J (2004) Genetic association of yield with its component traits in a recombinant inbred line population of cotton. Euphytica 140:171–179

Wu XS, Wang ZH, Chang XP, Jing RL (2010) Genetic dissection of the developmental behaviours of plant height in wheat under diverse water regimes. J Exp Bot 61:2923–2937

Xu YF, An DG, Liu DC, Zhang AM, Xu HX, Li B (2012a) Mapping QTLs with epistatic effects and QTL × treatment interactions for salt tolerance at seedling stage of wheat. Euphytica 186:233–245

Xu YF, An DG, Liu DC, Zhang AM, Xu HX, Li B (2012b) Molecular mapping of QTLs for grain zinc, iron and protein concentration of wheat across two environments. Field Crop Res 138:57–62

Yang J, Hu CC, Hu H, Yu RD, Xia Z, Ye XZ, Zhu J (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24:721–723

Ye ZH, Wang JM, Liu Q, Zhang MZ, Zou KQ, Fu XS (2009) Genetic relationships among panicle characteristics of rice (*Oryza sativa* L.) using unconditional and conditional QTL analyses. J Plant Biol 52:259–267

Zhang D, Cheng H, Wang H, Zhang HY, Liu CY, Yu DY (2010) Identification of genomic regions determining flower and pod numbers development in soybean (*Glycine max* L.). J Genet Genomics 37:545–556

Zhang JA, Hao CY, Ren Q, Chang XP, Liu GR, Jing RL (2011) Association mapping of dynamic developmental plant height in common wheat. Planta 234:891–902

Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W (2006) Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor Appl Genet 113:33–38

Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. Genetics 141:1633–1639

Zhu J (1999) Mixed model approaches of mapping genes for complex quantitative traits. J Zhejiang Univ (Nat Sci) 33:327–335

Zhuang QS (2003) Chinese wheat improvement and pedigree analysis (in Chinese). Chinese Agricultural Press, Beijing