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QTL detection for wheat kernel size and quality and the responses of these traits to low nitrogen stress

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Abstract

Key message **QTLs for kernel characteristics and tolerance to N stress were identified, and the functions of ten known genes with regard to these traits were specified.**

Abstract Kernel size and quality characteristics in wheat (*Triticum aestivum* L.) ultimately determine the end use of the grain and affect its commodity price, both of which are influenced by the application of nitrogen (N) fertilizer. This study characterized quantitative trait loci (QTLs) for kernel size and quality and examined the responses of these traits to low-N stress using a recombinant inbred line population derived from Kenong 9204 × Jing 411. Phenotypic analyses were conducted in five trials that each included low- and high-N treatments. We identified 109 putative additive QTLs for 11 kernel size and quality characteristics and 49 QTLs for tolerance to *N* stress, 27 and 14 of which were stable across the tested environments, respectively. These QTLs were distributed across all wheat chromosomes except for chromosomes 3A, 4D, 6D, and 7B. Eleven QTL clusters that simultaneously affected kernel size- and quality-related traits were identified. At nine locations, 25 of the 49 QTLs for N deficiency tolerance coincided with the QTLs for kernel characteristics, indicating their genetic independence. The feasibility of indirect selection of a superior genotype for kernel size and quality under high-N conditions in breeding programs designed for a lower input management system are discussed. In addition, we specified the functions of *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, *TaCwi-A1*, *TaSus2*, *TaGS2-D1*, *PPO-D1*, *Rht-B1*, and *Ha* with regard to kernel characteristics and the sensitivities of these characteristics to N stress. This study provides useful information for the genetic improvement of wheat kernel size, quality, and resistance to N stress.

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Abbreviations

GPC   Grain protein content
WGC   Wet gluten content

DT   Dough tractility
TW   Test weight
ABS   Water absorption
ZEL   Zeleny sedimentation value
KH   Kernel hardness
KL   Kernel length
KW   Kernel width
KDR   Kernel diameter ratio
TKW   Thousand-kernel weight
KRT   Kernel-related trait
KRTDV Differences in the value for a kernel-related trait between the high-nitrogen and low-nitrogen treatments

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide, and it is used to make a variety of food products. Wheat, rice and corn provide more than 60 % of the calories and protein needed for human nutrition (Gill et al. 2004). Breeding new varieties of wheat with enhanced yield potential, high tolerance to biotic and abiotic stresses and improved processing and end-use quality are the three key aims of wheat breeding programs (Mann et al. 2009). Improvement of these traits, which are quantitatively inherited and significantly influenced by the environment, depends on a thorough understanding of their genetic basis.

Kernel size, including kernel weight and dimensions such as kernel length (KL) and kernel width (KW), has significant effects on both yield and quality (Campbell et al. 2001; Sun et al. 2009; Ramya et al. 2010; Tsilo et al. 2010; Cui et al. 2011, 2014b). A comparison of wheat grain yield and yield component selection has shown that selection for kernel weight could result in an increase in grain yield (Alexander et al. 1984). However, an increase in yield often results in a decrease in kernel quality parameters, such as a lower grain protein content (GPC), because of a dilution effect (Suprayogi et al. 2009). Notably, previous studies have shown that quantitative trait loci (QTLs) for yield are not always negatively correlated with quality (Sourdille et al. 2003; Huang et al. 2006; Kunert et al. 2007; Echeverry-Solarte et al. 2015). Therefore, knowledge of the genetic basis of kernel size and its correlation with quality is valuable for the genetic improvement of both yield and quality.

Characteristics that affect the processing and end-use of grain involve a complex group of traits collectively known as quality traits (Kunert et al. 2007). Of them, GPC, wet gluten content (WGC), dough tractility or extensibility (DT), test weight or hectoliter weight (TW), water absorption (ABS), the Zeleny sedimentation score (ZEL), and

kernel hardness (KH) are all important grain quality traits in bread wheat. GPC, particularly glutenin and gliadin content is controlled by a complex genetic system and is strongly affected by the environment. It is an important factor for pasta-making and baking quality and is strongly correlated with WGC, DT, ABS, and ZEL (Zanetti et al. 2001; Nelson et al. 2006; Suprayogi et al. 2009; Li et al. 2011). KH, one of the most important determinants of milling yield, has profound effects on the milling, baking and end-use quality of bread wheat (Li et al. 2009). Numerous studies have identified an association between high- and low-molecular-weight (HMW/LMW) glutenin loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*/*Glu-A3*, *Glu-B3*, and *Glu-D3*), and wheat quality, and these loci are located on the long/short arms of the homoeologous group 1 chromosomes (Perretant et al. 2000; Sourdille et al. 2003; Kuchel et al. 2006; Li et al. 2009; Mann et al. 2009; Suprayogi et al. 2009; Sun et al. 2010; Reif et al. 2011; Echeverry-Solarte et al. 2015). In addition, the *Ha* locus on chromosome 5DS and the *Gpc-B1* locus on chromosome 6BS have also been reported to exhibit strong correlations with processing and end-use quality (Sourdille et al. 1996, 2003; Perretant et al. 2000; Campbell et al. 2001; Igrejas et al. 2002; Turner et al. 2004; Nelson et al. 2006; Kunert et al. 2007; Li et al. 2009; Sun et al. 2010). However, the majority of the reports described above have also shown that genetic factors influencing processing and end-use quality in both cultivars and in wild wheat are distributed over all 21 wheat chromosomes.

An adequate quantity of nitrogen (N) and an appropriate time of application can improve yield and quality; however, the use of N fertilizer leads to an increased cost as well as environmental consequences, such as groundwater pollution due to nitrate leaching (Laperche et al. 2007, 2008; Suprayogi et al. 2009; Cui et al. 2014a). Wheat varieties that maintain a high yield and quality under moderate or even severe N deficiency can be adapted for use in low-input management systems. An understanding of the genetic basis of N deficiency tolerance is essential for the breeding of such varieties. However, few QTLs for both yield/quality and their responses to N deficiency in wheat under field conditions have been documented to date (Habash et al. 2007; Laperche et al. 2007, 2008; Cui et al. 2014a). Favorable QTL alleles that decrease differences in yield and/or quality between high N (HN) and lower N (LN) conditions are of value in wheat breeding programs designed to increase N deficiency tolerance (Cui et al. 2014a).

Functional or gene-specific markers are derived from polymorphic sites within genes that are directly associated with phenotypic variations (Liu et al. 2012). In most cases, a functional marker of a gene co-localizes with the QTL peak position for the same trait (Sourdille et al. 2003). To date, numerous functional markers have been discovered

that facilitate analyses of the co-localization of identified QTLs with known genes (Giroux and Morris 1997; Lei et al. 2006; He et al. 2009; Wang et al. 2010; Jiang et al. 2011; Ma et al. 2012). Moreover, QTL analysis based on genetic mapping, including analysis of functional markers, can reveal novel functions of important genes that are indirectly correlated with yield and quality traits. In this study, we performed QTL analysis of 11 kernel characteristics under LN and HN conditions using a genetic map enriched with functional markers. The aims of this study were to (1) identify the genetic factors affecting kernel size and quality and the tolerances of these characteristics to N deficiency, (2) characterize the genetic relationships between kernel size and quality, and (3) specify the functions of important genes with regard to kernel characteristics and their sensitivities to N stress.

Materials and methods

Plant materials, experimental design, and phenotypic evaluation

The plant materials included a set of 188 recombinant inbred lines (RILs) derived from Kenong 9204 (KN9204) × Jing 411 (J411) (denoted by KJ-RIL) (Cui et al. 2014a; Fan et al. 2015). The 188 kJ-RILs and their parents were grown from 2011 to 2012 in Shijiazhuang (L1); 2012–2013 in Shijiazhuang (L2), Beijing (L3) and Xinxiang (L4); and 2013–2014 in Shijiazhuang (L5). An LN treatment and an HN treatment were applied in each trial for a total of ten environments (year × location × treatment), designated as E1, E2, E3, E4, E5, E6, E7, E8, E9, and E10. The N treatments, field arrangements and experimental designs of L1 to L4 are detailed in our previous reports (Cui et al. 2014a; Fan et al. 2015). T5 followed the above trial design and was carried out from 2013 to 2014 in Shijiazhuang.

Seven quality traits, including GPC, WGC, DT, TW, ABS, ZEL and KH, were measured by near-infrared reflectance spectroscopy (NIRS) with a Perten DA-7200 instrument (Perten Instruments, Huddinge, Sweden) and expressed on a 14 % moisture basis. The measurements were calibrated using calibration samples according to the manufacturer's instructions. Previous studies have confirmed that NIRS is a powerful method for measuring the quality traits of wheat; that it is rapid, effective, and convenient compared with traditional methods; and that it is appropriate for use in wheat breeding programs designed to improve quality-related traits (Sourdille et al. 1996; Perretant et al. 2000; Turner et al. 2004; Kuchel et al. 2006; Nelson et al. 2006; Li et al. 2009, 2012; Mann et al. 2009; Suprayogi et al. 2009; Sun et al. 2010; Asif et al. 2015).

The four kernel size-related traits were thousand-kernel weight (TKW), KL, KW, and kernel diameter ratio (KDR). These traits were evaluated after harvesting according to Cui et al. (2011). All lengths were reported in millimeters.

The differences in the values for the kernel-related traits (KRTs) between the HN and LN conditions in each trial were calculated as follows: $KRTDV = KRT_{(HN)} - KRT_{(LN)}$, where KRTDV is the difference in the value for a KRT for each line in each trial between the HN and LN conditions, and $KRT_{(HN)}$ and $KRT_{(LN)}$ represent the KRTs under HN and LN, respectively.

Statistical and QTL analyses

Analysis of variance (ANOVA) was performed and phenotypic correlation coefficients were calculated between traits and between N treatments (Spearman's rank correlation) using SPSS 19.0 (SPSS, Chicago, IL, USA; http://en.wikipedia.org/wiki/SPSS) and QGAStation 2.0 (http://ibi.zju.edu.cn/software/qga/v2.0/index_c.htm). To estimate the broad-sense heritability (h_B^2) of the corresponding traits, ANOVA was performed for each environment separately using QGAStation 2.0 according to Fan et al. (2015). The h_B^2 values were calculated using the following formula $h_B^2 = V_G/V_P$; where V_G and V_P are the genetic variance and phenotypic variance, respectively. To estimate the genotype × location interaction ($G \times L$) and the genotype × N treatment interaction effects ($G \times T$), a combined ANOVA over L1 to L5 was performed using SPSS 19.0. The genotypes (188 RILs), N treatments (LN and HN), and locations (L1, L2, L3, L4 and L5) were considered as fixed effects, whereas the blocks (two replications) were considered as random effects. F tests were considered significant at $p < 0.05$. Significant differences between genotypes, N treatments, and trials and their interaction effects were assessed by analyzing F-protected least significant difference (LSD) values at a significant level of $p < 0.05$. Correlations between two traits and Spearman's rank correlations between HN and LN were assessed by identifying significant Pearson's correlation coefficients at $p < 0.05$ using SPSS 19.0. The trait means for the five LN treatments (E1, E3, E5, E7, and E9) and the five HN treatments (E2, E4, E6, E8, and E10) were used to calculate phenotypic correlation coefficients between the traits under the LN and HN conditions, respectively. The 188 KJ-RILs were ranked according to the desired direction of selection for each trait based on the trait means for the five LN and five HN treatments. The numerical order was used to calculate Spearman's rank correlations cofficients between LN and HN.

A linkage map of the KJ-RIL population has been previously established (Cui et al. 2014a). In this study, 11 functional markers of kernel size and quality-related traits were used to saturate the KJ-RIL-derived genetic map, with the

aim of estimating whether the QTLs were co-localized with known genes. The 11 functional markers are related to the genes *Glu-B1*, *Glu-A3*, *cell wall-bound invertase* (*TaCwi-A1*), *sucrose synthase 2* (*TaSus2*), *Hardness* (*Ha*), and *phytoene synthase 1* (*Psy-B1*). Relevant information about these functional markers is listed in Supplementary Table S1. In addition, six *Rht*-linked markers were used to saturate the previously constructed map. Relevant information about these markers, along with QTLs for plant height-related traits, will be detailed in another paper. The new version of the genetic linkage map amended with the abovementioned functional and *Rht*-linked markers was used for QTL analysis.

The observed phenotypic values obtained from each environment (E1–E10) were used for QTL mapping analysis. Concerning KRTDV, the phenotypic values of the 188 RILs in L1, L2, L3, L4, and L5 and the means for the above five trials (M) were individually used in QTL mapping analysis. Inclusive composite interval mapping was performed using IciMapping 4.0 based on stepwise regression, with simultaneous consideration of all marker information (http://www.isbreeding.net/). The walking speed chosen for all QTLs was 1.00 cM, with a *p* value inclusion threshold of 0.001. The threshold for the detection of QTLs was calculated using 1,000 permutations, with a type 1 error of 0.05. All of the QTLs were designated according to Fan et al. (2015). A QTL with an average LOD value >3.0 and an average phenotypic variance contribution >10 % was defined as a major QTL, and one showing significance in at least five/three environments/data sets was considered a stable QTL for the 11 KRTs/KRTDVs.

Results

Phenotypic variation, performance, and correlations

Components of variance obtained by combined ANOVA were all highly significant for all traits. All of these traits, except for KW and KDR, showed significant $G \times L$ and $G \times T$ interaction effects. For GPC, WGC, ZEL, and KDR, the largest variances were caused by the N treatment (LN and HN), affecting DT, TW, KH, KL, KW, and TKW, and were influenced mainly by location. The highest variance in ABS was caused by a $G \times L$ interaction effect. Compared with the HN condition, the LN stress significantly reduced GPC, WGC, DT, ZEL, and KH but increased TW. The LN stress had inconsistent effects on ABS, KL, KW, KDR, and TKW across L1 to L5 (Supplementary Tables S1 and S2).

KN9204 performed better than J411 under both LN and HN for GPC, WGC, DT, ABS, ZEL and KH, with the exception of DT in E6. Conversely, J411 had a higher TW, KL and KDR than KN9204 under both LN and HN. In seven of the ten environments, the KW of KN9204 was slightly higher than that of J411. Conversely, J411 had a slightly higher TKW than KN9204 in seven of the ten environments (Supplementary Table S3). In nearly all cases, all 11 KRTs were quantitatively characterized with h_B^2 values ranging from 43.8 % (TW, 24.29–69.46 %) to 70.36 % (ABS, 56.65–88.66 %) (Supplementary information, Section 1.1; Supplementary Table S3).

Table 1 Phenotypic correlations among the 11 investigated traits in this study

	KL	KW	KDR	TKW	GPC	WGC	DT	TW	ABS	ZEL	KH
KL	**1.00**	0.08	0.61**	0.39**	0.13	0.15*	0.11	−0.13	0.10	0.08	0.09
KW	0.46**	**1.00**	−0.73**	0.71**	0.16*	0.18*	0.46**	0.43**	0.32**	0.12	0.33**
KDR	0.64**	−0.38**	**1.00**	−0.34**	−0.04	−0.04	−0.31**	−0.46**	−0.19*	−0.03	−0.20**
TKW	0.40**	0.80**	−0.27**	**1.00**	0.23**	0.26**	0.54**	0.42**	0.40**	0.19**	0.43**
GPC	0.12	0.10	0.06	0.09	**1.00**	0.98**	0.74**	−0.03	0.49**	0.53**	0.25**
WGC	0.16*	0.15*	0.07	0.13	0.98**	**1.00**	0.73**	−0.10	0.63**	0.54**	0.41**
DT	0.14*	0.25**	−0.05	0.31**	0.82**	0.81**	**1.00**	0.38**	0.49**	0.66**	0.45**
TW	−0.25**	0.12	−0.37**	0.22**	0.15*	0.05	0.37**	**1.00**	−0.11	−0.13	−0.12
ABS	0.21**	0.19**	0.08	0.25**	0.54**	0.68**	0.52**	−0.28**	**1.00**	0.44**	0.89**
ZEL	0.23**	0.08	0.19*	0.11	0.58**	0.63**	0.65**	−0.25**	0.69**	**1.00**	0.57**
KH	0.23**	0.20**	0.10	0.29**	0.27**	0.42**	0.36**	−0.41**	0.91**	0.74**	**1.00**

For each entry, the *top-right* cells show correlation coefficients at a high nitrogen (HN) level, and the *bottom-left* cells depict correlation coefficients at a low nitrogen (LN) level

GPC grain protein content, *WGC* wet gluten content, *DT* dough tractility, *TW* test weight, *ABS* water absorption, *ZEL* Zeleny sedimentation value, *KH* kernel hardness, *KL* kernel length, *KW* kernel width, *KDR* kernel diameter ratio, *TKW* thousand-kernel weight

** Correlation is significant at *p* < 0.01 level; * Correlation is significant at *p* < 0.05 level

The phenotypic correlations among the 11 traits are listed in Table 1. Of the four kernel size-related traits, both KL and KW were significantly positively correlated with TKW under both LN and HN, whereas KDR was significantly negatively correlated with TKW. All kernel quality-related traits, except for TW, were significantly positively correlated with each other. A comparison of the kernel size-related and kernel quality-related traits revealed that KL and WGC, KW and WGC, KW and DT, KW and ABS, KW and KH, KDR and TW, TKW and DT, TKW and TW, TKW and ABS, and TKW and KH were positively correlated with each other under both LN and HN. The remaining traits were inconsistently correlated with each other across the environments (Supplementary information, Section 1.1).

Spearman's rank correlation coefficients between the two N treatments were high for KL (0.80), TKW (0.79), KDR (0.75), and ABS (0.70), moderate for KH (0.60), KW (0.56), DT (0.57), GPC (0.52), and WGC (0.52), and low for TW (0.36) and ZEL (0.39). Direct selection under HN and LN (up to a 15 % selection intensity) resulted in the selection of 50 % or fewer RILs with similar GPC, TW, ZEL, and KH traits. For example, if the top nine GPC (at a 5 % selection intensity), 19 (at a 10 % selection intensity), and 28 (at a 15 % selection intensity) RILs for GPC in the population were selected for each N treatment, then only fewer than half (2, 8, and 9, respectively) had this trait in common when the selection was based on both treatments. Direct selection under HN and LN showed relatively high

consistency for the remaining seven traits investigated, especially for TKW and DT at selection intensities of 5, 10, and 15 % (Table 2; Fig. 1).

Genotype analysis of the ten genes related to kernel size and quality traits and a novel genetic linkage map

KN9204/J411 carried the null, 7 + 9, and 2 + 12/2*, 6 + 8, and 2 + 12 alleles at the three HMW glutenin loci. Concerning the three LMW glutenin loci, KN9204/J411 carried *Glu-A3b* or *Glu-A3d*/*Glu-A3a* or *Glu-A3c* and exhibited the loss of the *Glu-B3*/*Glu-B3h* alleles at *Glu-A3* and *Glu-B3*, respectively. The two parents had the same *Glu-D3* allele (Supplementary information, Section 1.2). Analysis of *CWI21* and *CWI22* revealed that KN9204 and J411 had the *TaCwi-A1b* and *TaCwi-A1a* alleles, which caused a decrease and increase in TKW, respectively. Analysis of *Sus2-SNP-185/589H2* showed that KN9204 and J411 had the *TaSus2* alleles, which resulted in an increase and decrease in TKW, respectively. Analysis of the functional markers of *Ha* revealed that KN9204 had the *Pinb-D1b* allele, whereas J411 had the *Pinb-D1a* allele. Further, analysis of *Psy-B1*, *YP7B-1* and *YP7B-2* revealed that KN9204 and J411 had the *Psy-B1b* and *Psy-B1c* alleles, respectively.
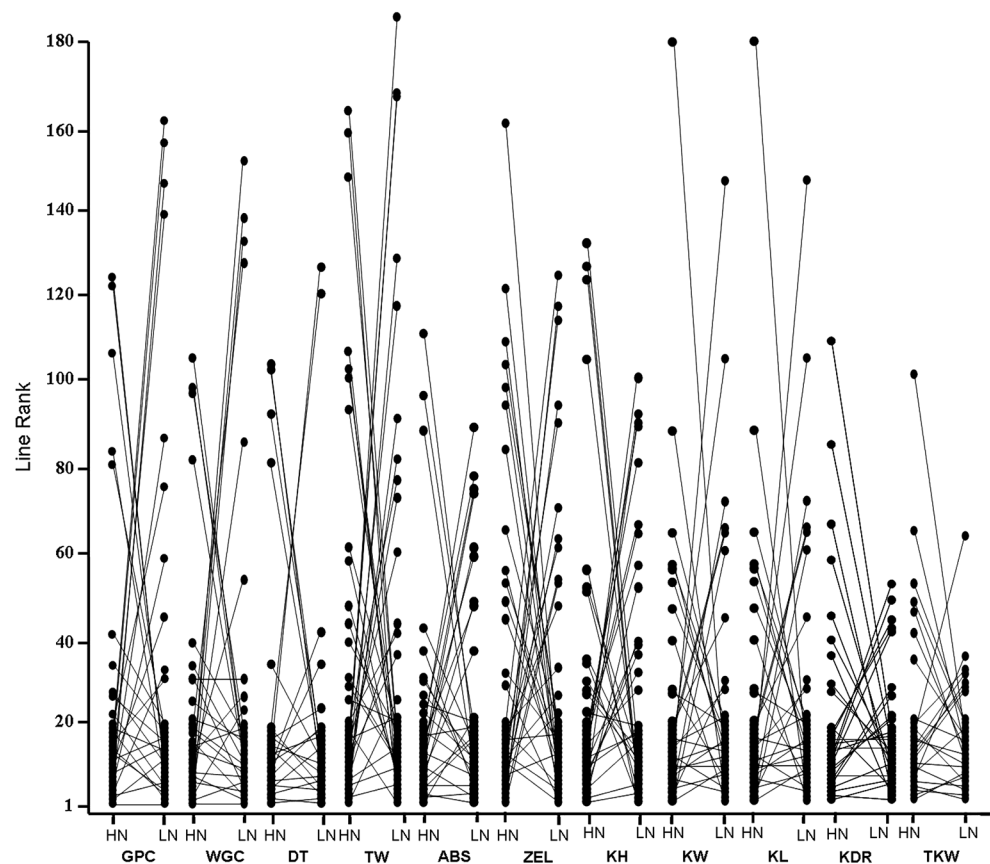
These functional markers were used to screen the 188 kJ-RILs and to perform linkage analysis to enrich the KJ-RIL map, and they were all assigned to the same chromosomes described in previous reports (Fig. S1). Due to the co-segregation of diagnostic markers for specific genes, the gene names [instead of its corresponding marker name (s)] were listed in the genetic map. In addition, six *Rht*-linked markers were added to the new version of the KJ-RIL map (data not shown). The novel genetic linkage map included 600 loci spanning 3947.6 cM with an average map density of one marker per 6.58 cM.

QTLs for the 11 KRTs and their responses to low N stress

A total of 109 and 49 QTLs were detected for the KRTs and KRTDVs, respectively. These QTLs were distributed across all wheat chromosomes except for 3A, 4D, 6D, and 7B. Of them, 74 QTLs were mapped to the B genome, 53 were mapped to the A genome, and 31 were mapped to the D genome (Supplementary Tables S4 and S5; Fig. 2). These QTLs individually explained an average of 2.85–35.57 % of the phenotypic variance, with average LOD values ranging from 2.07 to 18.23. Of the 109 QTLs for the 11 KRTs, 38 (34.86 %) were reproducibly detected in at least four of the ten environments (Table 3; Fig. 2). Of the 49 QTLs for the 11 KRTDVs, 14 (28.6 %) were reproducibly detected in at least three of the six data sets (L1, L2, L3, L4, L5, and M) (Table 4; Fig. 2).

Table 2 Spearman's rank correlations (r_s) along with the number of common recombinant inbred lines (RILs) at three selection intensities (5, 10, and 15 %) for 11 traits in the KJ-RIL population

Traits	r_s	Line selected in common		
		5 %[a]	10 %[a]	15 %[a]
		9[b]	19[b]	28[b]
GPC	0.52**	2	8	9
WGC	0.52**	5	10	14
DT	0.57**	5	13	16
TW	0.36**	2	4	8
ABS	0.70**	4	9	15
ZEL	0.39**	0	5	11
KH	0.60**	2	5	11
KL	0.80**	1	7	14
KW	0.56**	4	9	14
KDR	0.75**	4	10	14
TKW	0.79**	7	12	15

** Correlation is significant at $p < 0.01$ level

[a] Selection intensity applied within each nitrogen treatment

[b] Maximum number of lines selected from the experimental population of 188 RILs at the given selection intensity

QTLs for the 11 KRTs

Nine QTLs each for GPC, WGC, and DT; eight QTLs each for TW and ABS; and six QTLs each for ZEL and KH were identified. In addition, a total of 11, 13, 13, and 17 QTLs for KL, KW, KDR, and TKW were detected, respectively. These QTLs were distributed across 16 wheat chromosomes but were not found on 3A, 4D, 5A, 6D, or 7B. The favorable alleles for the ten QTLs for the KRTs (all but ZEL) were contributed by both KN9204 and J411. In addition, all six QTL alleles that increased ZEL were contributed by J411 (Table 3; Supplementary Table S4; Fig. 2; Supplementary information, Section 1.3).

QGpc-1A, *QGpc-1B.2*, *QWgc-1A*, *QWgc-1B.1*, *QWgc-5D*, *QDt-1A.2*, *QDt-2A*, *QDt-4A*, *QDt-4B*, *QDt-5D*, *QTw-4B.2*, *QAbs-1B.1*, *QAbs-3B*, *QAbs-5D*, *QZel-2A*, *QZel-5D*, *QKh-5D*, *QKl-1B*, *QKl-2A.1*, *QKw-2D*, *QKdr-2A*, *QKdr-2D*, *QKdr-4B.2*, *QTkw-2D*, *QTkw-4A*, *QTkw-4B.2*, and *QTkw-5D* were stable QTLs that were verified in at least five of the ten environments. Moreover, *QDt-4B*, *QTw-4B.2*, *QAbs-5D*, *QZel-5D*, *QKh-5D*, *QKl-1B*, and *QTkw-4B.2* were major stable QTLs. Additionally, there were 11 QTLs that were detected reproducibly in four of the ten environments (Table 3; Supplementary Table S4; Fig. 2).

The 27 stable QTLs were distributed among eight chromosomal regions. *QGpc-1A* and *QWgc-1A* were within a confidence interval near *Glu-A3* on chromosome 1A. *QWgc-1B.1* and *QAbs-1B.1* were within the confidence interval of *wPt-5312–Xme16em12.1* on chromosome 1B. *QGpc-1B.2* and *QKl-1B* co-localized on chromosome 1B in the vicinity of the known gene *Glu-B1*. *QDt-2A*, *QZel-2A*, *QKl-2A.1*, and *QKdr-2A*, were within a confidence interval near *TaCwi-A1* on chromosome 2A. *QKw-2D*, *QKdr-2D*, and *QTkw-2D* co-localized on chromosome 2D in the vicinity of the known genes *TaGS2-D1* and *PPO-D1*. *QDt-4A* and *QTkw-4A* were within the confidence interval of *Xgpw2331–Xwmc760* on chromosome 4A. *QDt-4B*, *QTw-4B.2*, *QKdr-4B.2*, and *QTkw-4B.2* were co-localized on chromosome 4B in the vicinity of the known gene *Rht-B1*. Finally, *QWgc-5D*, *QDt-5D*, *QAbs-5D*, *QZel-5D*, *QKh-5D*, and *QTkw-5D* were within a confidence interval near *Ha* on chromosome 5D (Table 3; Fig. 2).

QTLs for the 11 kernel-related traits with differences between HN and LN

A total of 49 QTLs were identified for the 11 KRTDVs that individually accounted for an average of 4.22–26.81 % of the phenotypic variation, with average LOD values of 2.07–8.57

Table 3 Putative additive QTLs for the 11 kernel-related traits, as identified by IciMapping 4.0, that showed consistency in no fewer than four of the ten different environments in the KJ population, in addition to QTLs in the same region reported in the literature

Trait	QTL[a]	Flanking markers	No. of environ-ment[b]	LOD[c]	PVE (%)[d]	Additive effect[e]	References documented the corresponding coincident QTLs[f]
GPC	*QGpc-1A*	*Xcnl137–BE425125*	6	2.87	6.43	0.23	1, 2
	QGpc-1B.2	*Glu-B1–Xme23em15.1*	5	3.21	7.29	0.23	1, 2, 3, 4, 5, 6, 7, 8
	QGpc-2A	*Xwmc522–TaCwi-A1*	4	3.18	7.53	−0.21	1, 6, 7, 9, 10
	QGpc-2B	*Xksum053–Xcfe212*	4	3.66	8.08	−0.20	11
	QGpc-4B	*Rht-B1–Xmag4087*	4	3.18	6.87	−0.27	12
WGC	*QWgc-1A*	*Xcnl137–BE425125*	5	2.80	6.25	0.58	
	QWgc-1B.1	*Xgwm374–Xissr811.3*	5	3.11	6.94	0.58	
	QWgc-1B.2	*Glu-B1–wPt-2315*	4	3.41	7.97	0.68	6
	QWgc-2A	*Xwmc522–Xme9em20*	4	2.73	5.99	−0.47	6
	QWgc-5D	*Ha–Xcfd18*	5	3.78	7.35	0.53	
DT	*QDt-1A.2*	*wPt-6046–Xwmc312*	6	4.05	7.23	−2.21	3, 4, 5, 13
	QDt-2A	*Xcfe67–Xme9em20*	8	2.98	6.65	−2.15	
	QDt-4A	*Xgpw2331–Xgpw7543*	5	2.76	4.92	1.86	
	QDt-4B	*Rht-B1–Xmag4087*	9	**6.03**	**11.44**	−3.10	
	QDt-5D	*Ha–Xcfd18*	8	4.83	8.02	2.27	3, 4
	QDt-7D	*Xcfd5–Xwmc824*	4	2.66	7.03	2.15	
TW	**QTw-4B.2**	*Rht-B1–Xmag4087*	6	**5.61**	**11.91**	−3.26	12
ABS	*QAbs-1B.1*	*wPt-5312–Xwmc402.2*	9	6.17	8.95	0.77	
	QAbs-2B	*Xwmc154–Xksum053*	4	2.99	4.75	−0.57	6, 10
	QAbs-3B	*Xwmc777–Xme12em20.1*	6	3.02	4.30	0.52	10
	QAbs-5D	*Ha–Xcfd18*	10	**18.23**	**32.75**	1.43	6, 13, 14
ZEL	*QZel-2A*	*Xwmc598–Xbarc89*	8	3.70	9.19	−1.91	6
	QZel-5D	*Ha–Xcfd18*	8	**7.71**	**16.54**	2.54	6, 9, 13, 15, 16
KH	*QKh-2D.1*	*XwmpE08–Xcfd233*	4	3.43	6.45	−1.23	10
	QKh-5D	*Ha–Xcfd18*	10	**18.14**	**35.57**	2.96	3, 4, 6, 11, 12, 13, 14, 16, 17, 18
KL	**QKl-1B**	*wPt-2315–Xwmc766*	7	**6.33**	**13.47**	−0.105	
	QKl-2A.1	*Xwmc522–Xksum052*	6	3.96	8.04	−0.080	
KW	*QKw-2D*	*XwmpE08–Xgpw5215.1*	9	4.68	9.05	0.049	19, 20, 21
	QKw-4B.2	*Xbarc199–Xmag2055*	4	**6.61**	**11.66**	−0.055	7, 19
	QKw-6B	*Xcnl64–Xcau10*	4	**4.88**	**13.00**	−0.060	
KDR	*QKdr-2A*	*Xme6em12–Xme9em20*	6	3.93	7.39	−0.027	21
	QKdr-2D	*Xswes61–Xmag4089*	6	4.12	7.26	−0.032	20
	QKdr-4B.2	*Xbarc199–Xcnl10*	5	**6.37**	**12.36**	0.044	7
TKW	**QTkw-2D**	*Xcfd233–Xgpw5215.1*	10	**5.58**	**10.16**	1.41	2, 19, 20, 21, 22, 23, 24, 25, 26
	QTkw-4A	*Xgpw2331–Xwmc760*	8	4.81	8.87	1.30	20, 21
	QTkw-4B.2	*Rht-B1–Xmag2055*	5	**7.99**	**13.85**	−1.84	7, 12, 20, 21, 23, 27, 28, 29, 30, 31
	QTkw-5D	*Ha–Xcfd18*	5	2.84	5.01	1.10	20
	QTkw-6A	*Xwmc553–Xwmc754*	4	2.42	4.15	−0.98	15

[a] A putative major QTL is marked in *bold* typeface and is characterized by a mean LOD > 3.0 and a mean PVE > 10 %, and a putative stable QTL is *underlined* when this locus was detected in at least five of the ten environments

[b] The number of environments in which the corresponding QTL showed significance

[c] The average LOD value across environments

[d] The average percentage of phenotypic variation explained by the QTL across environments

[e] A positive sign indicates that the alleles from the Kenong9204 parent increased the corresponding trait value, and a negative sign indicates that the alleles from the Jing411 parent increased the corresponding trait value

[f] *1* Suprayogi et al. (2009), *2* Echeverry-Solarte et al. (2015), *3* Perretant et al. (2000), *4* Sourdille et al. (2003), *5* Kuchel et al. (2006), *6* Li et al. (2009), *7* Mann et al. (2009), *8* Reif et al. (2011), *9* Breseghello et al. (2005), *10* Li et al. (2012), *11* Turner et al. (2004), *12* Sun et al. (2010), *13* Nelson et al. (2006), *14* Campbell et al. (2001), *15* Huang et al. (2006), *16* Kunert et al. (2007), *17* Sourdille et al. (1996), *18* Igrejas et al. (2002), *19* Ramya et al. (2010), *20* Cui et al. (2011), *21* Cui et al. (2014b), *22* Ammiraju et al. (2001), *23* Cuthbert et al. (2008), *24* Wang et al. (2009), *25* Gegas et al. (2010), *26* Zhang et al. (2015), *27* Huang et al. (2003), *28* Huang et al. (2006), *29* McCartney et al. (2005), *30* Zheng et al. (2010), *31* Wang et al. (2011)
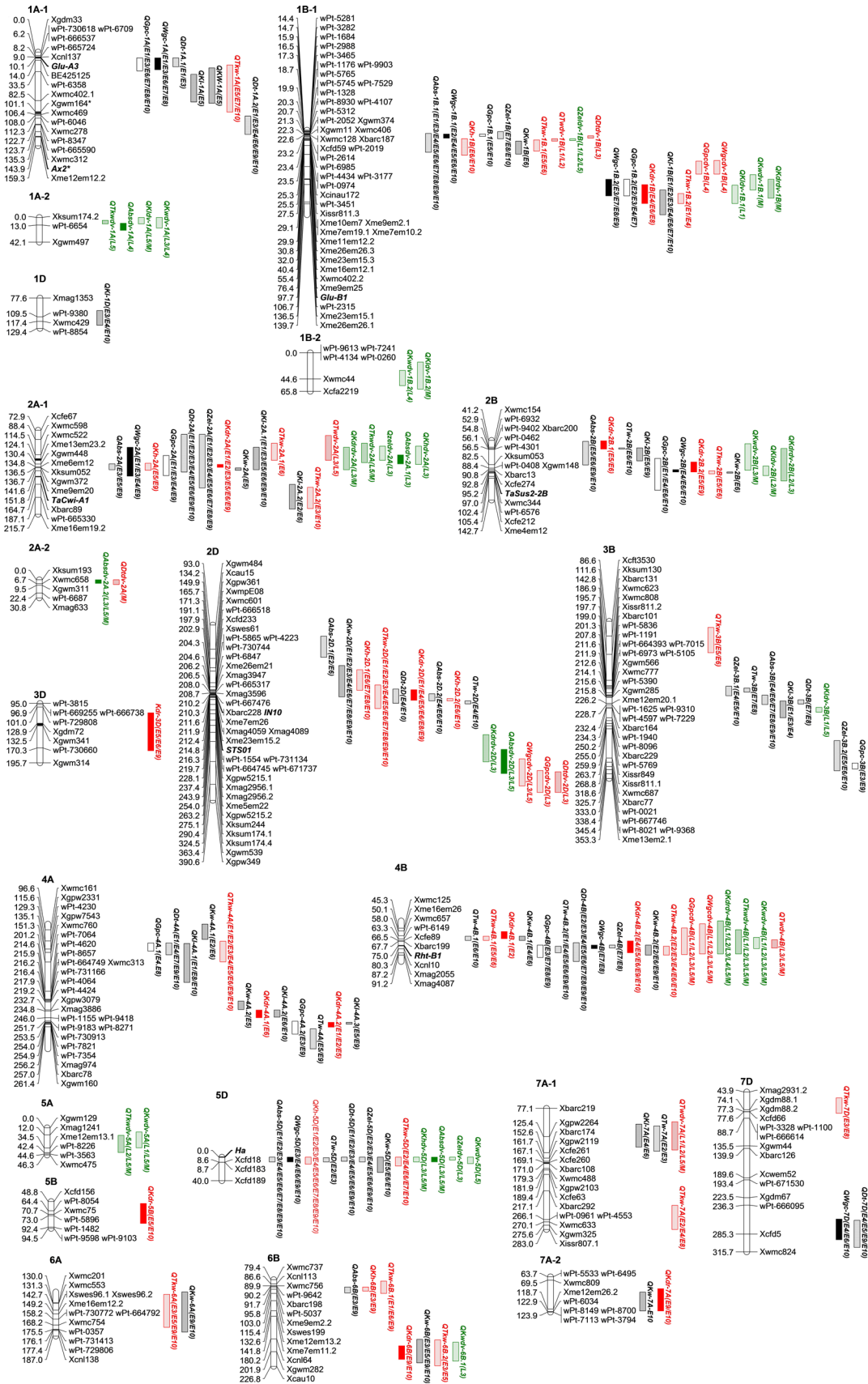
◄ **Fig. 2** Locations of QTLs for the 11 kernel size- and quality-related traits identified in ten individual environments based on a population of 188 kJ-RILs derived from a cross between Kenong 9204 and Jing 411. The short arms chromosomes are located at the top of the figure. The names of the marker loci and the QTLs are listed to the right of the corresponding chromosomes. The environments where the corresponding QTLs were detected are shown in parenthesis. The functional markers or gene names are marked by *bold italic* typeface. The positions of the marker loci are listed to the left of the corresponding chromosomes. The QTL intervals were associated with a LOD > 2.0, with LOD peak values of more than 2.5

(Supplementary Table S5; Fig. 2). These QTLs were mapped to chromosomes 1A (4 QTLs), 1B (10 QTLs), 2A (8 QTLs), 2B (3 QTLs), 2D (5 QTLs), 3B (1 QTL), 4B (6 QTLs), 5A (2 QTLs), 5D (4 QTLs), 6B (5 QTLs), and 7A (1 QTL). Twenty-two and 27 of these QTL alleles that reduced the KRTDVs were from KN9204 and J411, respectively.

Fourteen stable QTLs are listed in Table 4. Of them, *QGpcdv-4B*, *QWgcdv-4B*, *QTwdv-4B*, *QKwdv-4B*, *QKdrdv-4B*, and *QTkwdv-4B* were within the confidence interval on chromosome 4B near *Rht-B1*. All of these QTLs were major stable QTLs, except for *QTwdv-4B*. *QKwdv-5A* and *QTkwdv-5A* were within the confidence interval on chromosome 5A. *QAbsdv-5D* and *QKhdv-5D* were co-localized on chromosome 5DS in the vicinity of the known gene *Ha*. The remaining four stable QTLs were located on chromosomes 1B, 2A, 6B, and 7A (Table 4; Fig. 2).

Coincident QTLs for the KRTs and their responses to low-N stress

Twenty-five of the 49 QTLs for the KRTDVs coincided with the QTLs for the corresponding KRTs at nine locations on chromosomes 1B, 2A, 2B, 3B, 4B, 5D, 6B, and 7A (Table 5; Fig. 2). The *Xbarc199–Xmag4087* interval (near *Rht-B1*) harbored six pairwise QTLs for KRTs and KRTDVs associated with the traits GPC, WGC, TW, KW, KDR, and TKW. At this chromosomal region, J411 contributed to a higher GPC, WGC, TW, KW, and TKW and to reduced responses of GPC, WGC, and KDR to LN stress, and the KN9204 alleles in this region increased KDR and enhanced the tolerances of TW, KW, and TKW to LN stress. On chromosome 2A, five pairwise QTLs for KRTs and KRTDVs associated with the traits ABS, ZEL, KH, KDR, and TKW were mapped to *Xwmc598–Xbarc89*. J411 alleles in this chromosomal region contributed to the higher values of all five of these traits and also promoted the enhanced tolerance of ABS, ZEL, and KH and to the reduced tolerance of KDR and TKW to LN stress. Four pairwise QTLs for KRTs including GPC, WGC, KL, and KDR as well as the associated KRTDVs co-segregated with the known gene *Glu-B1* on chromosome 1B, with alleles from KN9204 increasing the tolerance of all four traits to LN stress. In this chromosomal region, KN9204 contributed to a higher GPC and WGC and J411

contributed to increased KL and KDR. Four pairwise QTLs for KRTs including ABS, ZEL, KH, and KW as well as the associated KRTDVs co-segregated with the known gene *Ha* on chromosome 5D, and KN9204 alleles in this region contributed to higher values for all four of these traits, in addition to the enhanced tolerance of ABS, ZEL, and KH to LN stress. *QKwdv-2B*, *QKdrdv-2B*, *QKw-2B*, and *QKdr-2B.2* were within the confidence interval of *wPt-9402–Xcfe212* on chromosome 2B. KN9204 and J411 alleles in this chromosomal region contributed to a greater KW and KDR, respectively; inversely, alleles that enhanced the tolerances of KW and KDR to LN stress were derived from J411 and KN9204, respectively. One QTL each for KL, KW, and TKW coincided with a QTL for KLDV, KWDV, and TKWDV, respectively, at three locations on chromosomes 3B, 6B, and 7A. J411 contributed to increased KL, KW, and TKW and to decreased KLDV, KWDV, and TKWDV in these chromosomal regions. On chromosome 1B, *QZel-1B* coincided with *QZeldv-1B*, and alleles from KN9204 enhanced the tolerance of ZEL to LN stress but reduced the ZEL.

Coincident QTLs for the kernel size- and quality-related traits

Eleven QTL clusters that simultaneously affected kernel size- and quality-related traits were identified on chromosomes 1B (two QTL clusters), 2A, 2B, 2D, 3B, 4A (two QTL clusters), 4B, 5D, and 6B, which harbored 28 QTLs for kernel size-related traits and 39 for kernel quality-related traits (Table 6; Fig. 2). All 11 QTL clusters, except for C4, C8, and C11, harbored major or stable or major stable QTLs for the KRTs. KN9204- or J411-derived alleles in C3, C5, C7, C9, and C11 that enhanced the kernel quality-related traits were also found to increase kernel size. In C1 and C10, KN9204 contributed to increased values for both kernel quality-related traits and to kernel size-related traits, with the exception of TW. In C2, alleles from KN9204 had additive effects on increases in the values for kernel quality-related traits and decreases in kernel size. In C4 and C8, alleles from J411 had additive effects on increases in the values of kernel quality-related traits and decreases in kernel size. In C6, KN9204 contributed to increased ABS and DT but to decreased ZEL, TW, and KL.

Discussion

Genetic relationships between kernel size and quality traits

In addition to productive spikes per unit area, kernels per spike, and kernel weight, kernel size is another major determinant of wheat yield (Cui et al. 2014b). The

Table 4 Putative additive QTLs for the differences between the values for 11 kernel-related traits under HN and LN, as identified by IciMapping 4.0, that showed consistency in no less than three of the six data sets for the KJ population

Trait	QTL[a]	Flanking markers	No. of data sets[b]	LOD[c]	PVE (%)[d]	Additive effect[e]
GPCDV	**_QGpcdv-4B_**	_Xwmc125–Xmag2055_	5	3.22	10.04	0.24
WGCDV	**_QWgcdv-4B_**	_Xwmc125–Xmag2055_	5	3.01	10.36	0.57
TWDV	_QTwdv-4B_	_Xbarc199–Rht-B1_	3	3.52	8.29	−2.56
	QTwdv-7A	_Xbarc219–Xbarc174_	4	2.22	8.11	2.84
ABSDV	_QAbsdv-2A.2_	_Xksum193–Xwmc658_	3	3.55	8.33	0.98
	QAbsdv-5D	_Ha–Xcfd18_	3	3.83	9.32	−0.48
ZELDV	_QZeldv-1B_	_Xme11em12.2–Xme26em26.3_	4	2.96	7.48	−1.67
KHDV	_QKhdv-5D_	_Ha–Xcfd18_	3	2.87	7.48	−1.10
	QKhdv-6B	_Xedm149.2–wPt-3045_	4	2.40	5.86	1.12
KWDV	**_QKwdv-4B_**	_Xwmc657–Xcnl10_	5	5.68	12.82	−0.058
	QKwdv-5A	_Xmag1241–Xme12em13.1_	3	2.47	7.89	−0.038
KDRDV	**_QKdrdv-4B_**	_Xbarc199–Xmag2055_	6	5.39	11.01	0.035
TKWDV	**_QTkwdv-4B_**	_Xme16em26–Xmag2055_	5	6.76	14.99	−1.31
	QTkwdv-5A	_Xmag1241–Xme12em13.1_	3	4.67	11.77	−1.24

[a] A putative major QTL is marked in _bold_ typeface and is characterized by a mean LOD > 3.0 and a mean PVE > 10 %, and a putative stable QTL is _underlined_ when this locus was detected in at least three of the six data sets

[b] The number of data sets where the corresponding QTL showed significance

[c] The average LOD value across data sets

[d] The average percentage of explained phenotypic variation by the QTL across data sets

[e] A positive sign indicates that the alleles from the Kenong9204 parent increased the difference in the corresponding trait value between HN and LN, and a negative sign indicates that the alleles from the Jing411 parent increased the difference in the corresponding trait value between HN and LN

negative yield-quality correlation has long been a key obstacle in wheat breeding programs designed to simultaneously improve yield and quality (Bathia and Rabson 1987; Nelson et al. 2006; Kunert et al. 2007; Suprayogi et al. 2009). Consequently, QTLs involved in the control of kernel quality that are also independent of negative effects on grain yield should be identified to improve this characteristic.

In this study, TKW showed significant positive correlations with DT, TW, ABS, and KH under LN and HN. It also exhibited positive correlations with GPC, WGC, and ZEL, although these correlations were non-significant under LN (Table 1). These findings imply that common genetic factors that simultaneously cause increase in TKW and enhancement of quality traits exist in KN9204 and/or J411. Five such QTL clusters were identified in C3, C5, C7, C9, and C11 (Table 6; Fig. 2). These chromosomal regions should be valuable for the genetic improvement of both kernel size and quality. For example, in the QTL cluster of C3, J411-derived alleles should be preferably selected for the simultaneous genetic improvement of ABS, WGC, KH, GPC, DT, ZEL, KDR, KW, KL, and TKW (Table 6). The ten markers in the _Xwmc598–Xbarc89_ interval should be used in the foreground selection. Thus, these QTL clusters should be precisely mapped and cloned to identify markers for more efficient selection.

Five chromosomal regions on chromosomes 1A (two regions), 2D, 3B, and 7D harbored QTLs that only affected kernel quality (Supplementary Table S4; Fig. 2). Eight chromosomal regions harbored QTLs that only affected the kernel size and were distributed among chromosomes 1A, 3B, 3D, 4A, 6A, 7A (two regions) and 7D (Supplementary Table S4; Fig. 2). These findings indicate that kernel size and the quality traits are under independent genetic control in some cases. Thus, these chromosomal regions should be valuable for the genetic improvement of quality/kernel size without influencing kernel size/quality traits. Further, some of these QTLs were stable across the tested environments, for example, the QTLs _QGpc-1A_ and _QWgc-1A_, which had overlapping confidence intervals on chromosome 1A and were significant in six and five environments, respectively. Such stable QTLs are of particular value for marker-assisted selection in the breeding of varieties adapted to various ecological environments.

Would indirect selection of superior genotypes under normal conditions maximize the genetic gain in breeding programs designed to improve tolerance to N-deficient conditions?

Indirect selection of superior genotypes in different systems will not always result in selection of the best possible

Table 5 Chromosomal regions simultaneously affecting a kernel-related trait and the difference in the corresponding value under HN and LN in this study

Chromosome	Interval	QTL for KRTDVs	Additive effect	QTL for KRTs	Additive effect
1B	*wPt-5765–Xme26em26.3*	*QZeldv-1B*	−1.67	*QZel-1B*	−1.68
	Xme9em25–Xme23em15.1	*QGpcdv-1B*	−0.06	*QGpc-1B.2*	0.23
		QWgcdv-1B	−0.16	*QWgc-1B.2*	0.68
		QKldv-1B.1	−0.064	*QKl-1B*	−0.105
		QKdrdv-1B	−0.019	*QKdr-1B*	−0.047
2A	*Xwmc598–Xbarc89*	*QAbsdv-2A.1*	0.50	*QAbs-2A*	−0.47
		Qzeldv-2A	1.68	*QZel-2A*	−1.91
		QKhdv-2A	1.17	*QKh-2A*	−0.91
		QKdrdv-2A	−0.022	*QKdr-2A*	−0.027
		QTkwdv-2A	−0.81	*QTkw-2A.1*	−1.24
2B	*wPt-9402–Xcfe212*	*QKwdv-2B*	0.048	*QKw-2B*	0.038
		QKdrdv-2B	−0.029	*QKdr-2B.2*	−0.017
3B	*Xgwm285–wPt-8096*	*QKldv-3B*	0.060	*QKl-3B*	−0.092
4B	*Xbarc199–Xmag4087*	*QGpcdv-4B*	0.24	*QGpc-4B*	−0.18
		QWgcdv-4B	0.57	*QWgc-4B*	−0.84
		QTwdv-4B	−2.56	*QTw-4B.2*	−3.26
		QKwdv-4B	−0.058	*QKw-4B.2*	−0.055
		QKdrdv-4B	0.035	*QKdr-4B.2*	0.044
		QTkwdv-4B	−1.31	*QTkw-4B.2*	−1.84
5D	*Ha–Xcfd183*	*QAbsdv-5D*	−0.48	*QAbs-5D*	1.43
		QZeldv-5D	−1.48	*QZel-5D*	2.54
		QKhdv-5D	−1.10	*QKh-5D*	2.96
		QKwdv-5D	0.042	*QKw-5D*	0.044
6B	*Xcnl64–Xcau10*	*QKwdv-6B.1*	0.057	*QKw-6B*	−0.060
7A	*Xbarc219–Xbarc174*	*QTwdv-7A*	2.84	*QTw-7A*	−2.78

KRTs kernel-related traits, *KRTDVs* the differences in the values for the KRTs between HN and LN for each line in each trial

Table 6 QTL clusters simultaneously affecting kernel size and quality-related traits in this study

Cluster	Chromosome	Interval	No. of QTLs	Traits (additive effect, number of environments)[a]
C1	1B	*Xgwm374–Xwmc402.2*	7	<u>ABS(+, 9)</u>, <u>WGC(+, 5)</u>, KH(+, 2), GPC(+, 2), ZEL(−, 3), **KW(+, 1)**, TKW(+, 2)
C2	1B	*Glu-B1–Xwmc766*	5	WGC(+, 4), <u>GPC(+, 5)</u>, KDR(−, 3), **<u>KL(−, 7)</u>**, TKW(−, 2)
C3	2A	*Xwmc598–Xbarc89*	10	ABS(−, 2), WGC(−, 4), KH(−, 2), GPC(−, 4), <u>DT(−, 8)</u>, <u>ZEL(−, 8)</u>, <u>KDR(−, 6)</u>, **KW(−, 1)**, <u>KL(−, 6)</u>, TKW(−, 1)
C4	2B	*wPt4301–Xcfe212*	5	GPC(−, 4), WGC(−, 3), KW (+, 1), KDR(−, 2), TKW(+, 2)
C5	2D	*XwmpE08–Xgpw5215.1*	7	ABS(+, 3), KH(+, 2), TW(+, 2), DT(+, 2), <u>KW(+, 9)</u>, <u>KDR(−, 6)</u>, **TKW(+, 10)**
C6	3B	*Xbarc101–wPt-8096*	5	ZEL(−, 3), TW(−, 2), <u>ABS(+, 6)</u>, DT(+, 2), KL(−, 3)
C7	4A	*Xwmc161–Xgpw7543*	5	GPC(+, 2), <u>DT(+, 5)</u>, KW(2, +), KL(+, 3), <u>TKW(+, 8)</u>
C8	4A	*wPt-8271–Xmag1140*	4	GPC(−, 2), TW(−, 2), KL(+, 2), KDR(+, 3)
C9	4B	*Xbarc199–Xmag2055*	8	GPC(−, 4), **<u>TW(−, 6)</u>**, **<u>DT(−, 9)</u>**, WGC(−, 2), ZEL(−, 2), **<u>KDR(+, 5)</u>**, **KW(−, 4)**, **<u>TKW(−, 5)</u>**
C10	5D	*Ha–Xcfd189*	8	**<u>ABS(+, 10)</u>**, <u>WGC(+, 5)</u>, **<u>KH(+, 10)</u>**, TW(−, 2), <u>DT(+, 8)</u>, **<u>ZEL(+, 8)</u>**, KW(+, 3), <u>TKW(+, 5)</u>
C11	6B	*Xcnl113–Xme9em2.2*	3	ABS(−, 2), KH(−, 2), TKW(−, 3), KDR(+, 2)

[a] A trait name in *bold type* indicates that major QTLs were detected for the corresponding trait, and a trait name in *underlined type* indicates that stable QTLs were detected for the corresponding traits

genotypes for improving stress resistance in breeding programs. A recent study has shown that direct selection in a conventional and organic management system (selection intensity of up to a 15 %) resultes in the selection of 50 % or fewer RILs with common yield- and quality-related traits (Asif et al. 2015). Ceccarelli and Grando (1991) have reported that the best lines selected in low-yield environments outperform superior lines selected in high-yield environments. In addition, the indirect selection efficiency of superior genotypes is trait dependent, and indirect selection can lead to greater genetic gains than direct selection for highly heritable traits (Hill et al. 1998; Asif et al. 2015).

In this study, the Spearman's rank correlation coefficient between the treatments for TKW was second to that for KL among the 11 traits investigated (Table 2; Fig. 1). Indirect selection for TKW can maximize genetic gain for the improvement of N stress tolerance. Similar inferences were also made for KL, KW, and KDR because they showed strong correlations with TKW. For DT and WGC, indirect selection under HN allowed for the genetic improvement of DT and WGC under LN in most cases; however, the negative effects of N stress on both DT and WGC were difficult to counteract. Indirect selection under HN allowed for the genetic improvements of ABS under LN to some extent, and it resulted in lower efficiencies for the genetic improvement of GPC, TW, ZEL, and KH under LN (Supplementary information, Section 2.1).

The environments in which a QTL is expressed determine the manner by which breeders use the corresponding QTL in molecular breeding programs, especially for stable QTLs. All the 27 stable QTLs for the 11 traits were expressed in both N environments (Supplementary Table S4; Fig. 2). This finding indicates that marker-assisted selection using the flanking markers of these QTLs is efficient under both HN and LN conditions. However, three of these stable QTLs—*QWgc-1B.1*, *QTkw-4B.2*, and *QTkw-5D*—were mainly expressed under HN condition; therefore, the flanking markers of these QTLs are useful for breeding wheat lines that can be grown under optimal N conditions in most cases. Of the 82 environment-specific QTLs, 17 and 23 were expressed only under LN and HN conditions, respectively, and the remaining 42 environment-sensitive QTLs were expressed under both N conditions (Supplementary Table S4; Fig. 2). *QGpc-2B*, *QWgc-2B*, *QWgc-7D*, *QAbs-2D.2*, *QKw-4B.2*, and *QKdr-1B* were significant in three of the five HN environments, and these six QTLs are useful for breeding wheat for growth under optimal N conditions. *QGpc-4B*, *QWgc-1B.2*, *QWgc-2A*, *QAbs-2A*, *QKw-6B*, and *QTkw-6A* were significant in three of the five LN environments, and these six QTLs are useful for breeding wheat for growth under N-stress environments.

Genomic regions conferring tolerance to low-N stress and their value in wheat breeding programs

Wheat varieties that maintain yield and quality under N stress can adapt to low-input management systems (Cui et al. 2014a). The examination of genetic variation in adaptive traits for N deficiency is essential for the breeding of varieties with N stress tolerate. However, few QTLs for yield and quality and their responses to N deficiency in wheat under field conditions have been documented (Habash et al. 2007; Laperche et al. 2007, 2008; Cui et al. 2014a).

QTL alleles enhance both KRTs and their tolerances to N stress should be valuable in wheat molecular breeding programs. In this study, 25 QTLs for KRTDVs were within the confidence intervals of the QTLs for the corresponding KRTs (Table 5; Fig. 2). Of them, 13 pairwise QTLs for KRTDVs and the corresponding KRTs showed opposite additive effects, indicating that these regions harbor genes that simultaneously enhance KRTs and their N stress tolerance. Interestingly, *QAbsdv-5D*, *QAbs-5D*, *QKhdv-5D*, and *QKh-5D* all mapped to locations near the *Ha* locus, and they were all stable across the tested environments. These findings indicated that the *Ha* locus tended to have stable pleiotropic effects on both the kernel quality-related traits (especially KH and ABS) and their responses to N stress. *QGpcdv-4B*, *QGpc-4B*, *QWgcdv-4B*, and *QWgc-4B* all mapped to locations near *Rht-B1*, and each was identified in at least four environments, except for *QWgc-4B*, which was significant in two environments. These findings indicated that the *Rht-B1* affected not only plant height but also grain quality and its adaptability to N-deficient environments. *QTwdv-7A* and *QTw-7A* both mapped to *Xbarc219–Xbarc174* on chromosome 7A and were verified in four of the six data sets and in two of the ten environments, respectively. Thus, closely linked markers of these QTLs are of value for the genetic improvement of LN stress tolerance.

Stable QTLs for KRTDVs that have no direct effects on the corresponding KRTs can also be used for the genetic improvement of LN stress tolerance. In this study, such stable QTLs were distributed among chromosomes 2A (*QAbsdv-2A.2*), 6B (*QKhdv-6B*), and 5A (*Kwdv-5A* and *Tkwdv-5A*) and were verified in three, four, three, and three data sets, respectively (Table 4). Of them, *Kwdv-5A* and *Tkwdv-5A* both mapped to *Xmag1241–Xme12em13.1* on chromosome 5A, and *Tkwdv-5A* was a major stable QTL individually accounting for an average of 11.77 % of the phenotypic variance, with an average LOD value of 4.67. Thus, markers that are closely linked with these QTLs could be used for the genetic improvement of these traits for LN stress tolerance.

Novel, stable QTLs and QTL co-segregation with known genes

Numerous genes associated with simply inherited traits have already been mapped, and corresponding functional makers have been developed (Liu et al. 2012). In most cases, the functional marker of a gene is located in very close proximity to the QTL for the same trait (Sourdille et al. 2003). The previous KJ-RIL-derived genetic map included seven functional markers, namely *Ax2** (*Glu-A1*), *FM1* (*UREG*), *Glu-b3h* (*Glu-B1*), *In10* (*Gs2-D1*), *PPO33* (*PPO-A1*), *STS01* (*PPO-D1*), and *FM2* (*PIP*) (Cui et al. 2014a). In this study, 11 functional markers of kernel size- and quality-related traits, along with six *Rht*-linked markers, were added to the new version of the KJ-RIL genetic map (Supplementary Fig. S1). These data can facilitate the determination of whether the QTLs for KRTs are co-localized with known genes.

Numerous studies have identified associations between HMW and LMW glutenin subunits and wheat quality (Payne 1987; Perretant et al. 2000; Sourdille et al. 2003; McCartney et al. 2006; Nelson et al. 2006; Zhang et al. 2008; Li et al. 2009; Mann et al. 2009; Suprayogi et al. 2009; Reif et al. 2011; Li et al. 2012; Echeverry-Solarte et al. 2015). In this study, KN9204 and J411 differed at two HMW glutenin loci (*Glu-A1* and *Glu-B1*) and two LMW glutenin loci (*Glu-A3* and *Glu-B3*). QTL mapping analysis showed that the 2*-HMW subunit encoded by the *Glu-1Ab* allele from J411 had a positive effect on DT; that the Bx7 + By9 allele from KN9204 could increase GPC and WGC, reduce kernel size, and increase the tolerance of GPC, WGC, KL, and KDR to LN stress; and that the *Glu-A3b* or *Glu-A3d* alleles from KN9204 could simultaneously increase GPC, WGC, and DT (Table 5). No adverse effects of the 1BL.1RS translocation on the quality trait were identified for *Glu-B3*, consistent with Johnson et al. (1999) (Supplementary information, Section 2.2).

The cell wall invertase (CWI) gene is associated with TKW (Ma et al. 2012; Liu et al. 2012; Rasheed et al. 2014). In this study, QTL mapping analysis showed that the *TaCwi-A1a* allele may be associated with not only an increased kernel size but also improved kernel milling and baking quality and that it could enhance the tolerance of both quality and yield to N stress. The sucrose synthase 2 (Sus2) gene is associated with wheat yield (Jiang et al. 2011; Liu et al. 2012; Rasheed et al. 2014). In this study, QTL mapping analysis showed that *TaSus2* had opposite effects on the yield and quality traits. In addition, *TaSus2* could influence the responses of KL and KW to N stress, but it had no effect on the response of per-plant yield (Cui et al. 2014a). Glutamine synthetase (GS2) is a key enzyme involved in plant N metabolism that is responsible for the first step of ammonium assimilation and transformation into glutamine; thus, it plays a key roles in the growth, N use and yield potential of cereal crops, including wheat (Gadaleta et al. 2011; Li et al. 2011). Polyphenol oxidase (PPO) activity is highly correlated with the undesirable browning of wheat-based end products, especially Asian noodles (He et al. 2007; Liu et al. 2012). *TaGS2-D1* and *PPO-D1* have been mapped to 210.30 and 214.80 cM on chromosome 2DL in the KJ-RIL map, respectively (Cui et al. 2014a). In this study, QTL mapping analysis showed that *TaGS2-D1a* from KN9204 had positive effects on KW, TKW, and KDR, and that *PPO-D1* from KN9204 had positive effects on DT and ABS. *QKh-2D.1* and *QTw-2D* might have been identified due to the presence of other linked genes. The *Rht-B1* gene is associated with physiological processes that affect not only plant height but also yield, grain quality, seedling vigor and adaptability to adverse environments (Fischer and Stockman 1986; Gooding et al. 1999, 2012; McCartney et al. 2006; Zheng et al. 2010; Bai et al. 2013; Zhang et al. 2013; Asif et al. 2015). This study indicated that *Rht-B1b* had negative effects on TKW, KW, KDR, TW, DT, and ZEL. The associations with *QGpc-4B* and *QWgc-4B* might have been due to the presence of other linked genes (Supplementary Fig. S2). In addition, *Rht-B1b* enhanced the tolerance of TW, KW, and TKW to N-deficiency (Table 5). KH, which has a profound effect on milling and end-use quality, is largely determined by the *Pina-D1* and *Pinb-D1* genes that encode the puroindoline *a* and puroindoline *b* proteins, respectively (Li et al. 2009; Liu et al. 2012). This study indicated that *Pinb-D1b* had pleiotropic effects that involved increasing KH and thus increasing WGC, DT, ABS, ZEL, KW, and TKW while decreasing TW, consistent with the additive effects listed in Table 6 (Supplementary Fig. S3). Moreover, the *Pinb-D1b* alleles from KN9204 enhanced the tolerance of KH, ABS, and ZEL to N deficiency but reduced the tolerance of KW (Table 5) (Supplementary information, Section 2.2).

No QTLs for kernel characteristics were mapped to areas near the remaining functional markers on the KJ-RIL genetic map, which might be attributed to the absence of associated genes affecting these traits in these regions. In addition, functional markers of several important major genes for grain shape and size including *GS3*, *GW2* and *GASR7*, did not show polymorphisms between the two parental lines (data not shown). We detected QTLs for kernel size on the corresponding chromosomes, including 6A, 7A, and 7D, which should have no associations with these genes. To infer whether common interacting QTLs or regions were presented across genetic backgrounds, we compared the results of our study with those of similar previous studies on wheat (Table 3). Of the 38 QTLs that were reproducibly detected in at least four of the ten environments, 26 had been reported in previous studies. All of the QTLs that were found to be located near the

abovementioned functional markers were QTLs or regions that commonly interacted across genetic backgrounds and have been reported in numerous similar studies (Table 3). In addition, *QWgc-1A*, *QWgc-1B.1*, *QAbs-1B.1*, *QKl-1B*, *QDt-2A*, *QKdr-2A*, *QKl-2A.1*, *QWgc-5D*, *QDt-4A*, *QDt-4B*, *QKw-6B*, and *QDt-7D* were novel QTLs that have not been reported previously, but some of them coincided with QTLs that interacted with other traits, indicating pleiotropic effects or the presence of linked genes. Interestingly, all of these novel QTLs except for *QKw-6B* and *QDt-7D* were stable across the environments, and both *QKl-1B* and *QDt-4B* were major stable QTLs. To our knowledge, this is the first study to report contributions of all of the QTLs for these 11 KRTs to N stress tolerance, and 14 of these QTLs were found to be stable across environments (Table 4). These findings should be valuable for the genetic improvement of both kernel characteristics and N-deficiency tolerance via marker-assisted selection.

In summary, this study has revealed the genetic factors affecting kernel size and quality and has provided the first report of QTLs associated with N-deficiency tolerance. A total of 109 putative additive QTLs for the 11 KRTs and 49 QTLs for the 11 KRTDVs were identified, 27 and 14 of which were stable across environments, respectively. Genetic relationships between kernel size and quality were well characterized at the QTL level. The feasibility of indirect selection of a superior genotype for kernel size and quality under normal N conditions in breeding programs designed for adaptation to a lower-input management system was discussed in depth. In addition, we specified the functions of genes important to kernel characteristics and the sensitivities of these characteristics to N stress. This study provides useful information for the genetic improvement of wheat kernel-related traits and the tolerance of wheat to N stress.

Author contribution statement Fa Cui, Xiaoli Fan, and Junming Li designed the study; Fa Cui, Xiaoli Fan, Chunhua Zhao, Na Zhang and Mei Chen conducted genotyping of the KJ-RIL population; Fa Cui, Xiaoli Fan, Mei Chen, Chunhua Zhao, Wei Zhang, Ji Jun, Jie Han, Xueqiang Zhao, Lijuan Yang, Yiping Tong, Zongwu Zhao, and Junming Li conducted phenotyping of the KJ-RIL population; Fa Cui analyzed the data and wrote the paper; and Junming Li and Tao Wang shared primary responsibility for the final content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards All of the authors have read and have abided by the statement of ethical standards for manuscripts submitted to *Theoretical and Applied Genetics*.

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