Molecular mapping of quantitative trait loci for kernel morphology traits in a non-1BL.1RS × 1BL.1RS wheat cross

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Abstract. The improvement of kernel morphology traits is an important goal in common wheat (\textit{Triticum aestivum} L.) breeding programs because of their close relationship with grain yield and milling quality. The aim of this study was to map quantitative trait loci (QTL) for kernel morphology traits using 240 recombinant inbred lines derived from a cross between the non-1BL.1RS translocation cv. PH 82-2 and the 1BL.1RS translocation cv. Neixiang 188, grown in six environments in China. Inclusive composite interval mapping identified 71 main-effect QTL on 16 chromosomes for seven kernel morphology traits measured by digital imaging, viz. kernel length, width, perimeter, area, shape factor, factor form-density and width/length ratio. Each of these loci explained from 2.6 to 28.2% of the phenotypic variation. Eight QTL clusters conferring the largest effects on kernel weight and kernel morphology traits were detected on chromosomes 1BL.1RS (2), 2A, 4A, 4B, 6B, 6D and 7A. Fourteen epistatic QTL were identified for all kernel morphology traits except kernel width/length ratio, involving 24 main-effect QTL distributed on 13 chromosomes, and explaining 2.5–8.3% of the phenotypic variance. Five loci, viz. \textit{Sec-1} on 1BL.1RS, \textit{Glu-B1} on 1BL, \textit{Xcfe53} on 2A, \textit{Xwmc238} on 4B, and \textit{Xbarc174} on 7A, were detected consistently across environments, and their linked DNA markers may be used for marker-assisted selection in breeding for improved wheat kernel traits and grain yield.

Additional keywords: kernel morphology traits, epistatic quantitative trait loci, main-effect quantitative trait loci, recombinant inbred lines, wheat.

Introduction

Kernel morphology traits are the major determinants of kernel weight and have a direct effect on grain yield (Campbell \textit{et al.} 1999; Gupta \textit{et al.} 2006; Bresegghello and Sorrells 2007). In conventional breeding applications, kernel morphology traits are usually evaluated by kernel weight, which may be correlated with several characters including kernel shape, kernel size and thickness (Giura and Saulescu 1996). Good kernel size, shape and uniformity are important criteria in determining market value or commercial success of improved wheat cultivars (Marshall \textit{et al.} 1986; Campbell \textit{et al.} 1999). Thus, understanding the genetic basis of kernel morphology traits is extremely important for wheat breeding programs.

Kernel morphology traits are also highly important milling traits in wheat (Marshall \textit{et al.} 1986; Berman \textit{et al.} 1996). Most

Abbreviations: FFD, factor form-density; G × E, genotype by environment interaction; ICIM, inclusive composite interval mapping; KA, kernel area; KW/L, kernel width/length ratio; KLEN, kernel length; KP, kernel perimeter; KWID, kernel width; NX, Neixiang 188; PH, PH 82-2; PV, phenotypic variance; PVE, phenotypic variance explained; QTL, quantitative trait loci; RIL, recombinant inbred line; SF, shape factor; TKW, one-thousand kernel weight.
breeders prefer plump kernels over long and thin kernels, because variation in kernel morphology traits determines quality characteristics set by the milling industry (Berman et al. 1996), and it may also have an effect on baking quality (Bergman et al. 2000). Up to 66% of the variation in milling yield could be predicted by kernel traits as measured by digital imaging, therefore, Berman et al. (1996) suggested that digital imaging of kernel morphology could be used to predict milling quality in breeding programs. Marshall et al. (1986) claimed that each kernel morphology trait made a minor but cumulative contribution to milling yield, although it seemed that kernel morphology traits were inherited independently of each other (Bergman et al. 2000; Breseghello and Sorrells 2007).

Generally, kernel morphology traits exhibit moderate to high broad-sense heritabilities ($h^2$) (Campbell et al. 1999; Sun et al. 2009; Gegas et al. 2010; Tsilo et al. 2010). Heritability estimates for large kernel size were higher than for small kernel size (Tsilo et al. 2010). Genotypic and environmental effects of kernel size and shape were found to differ across populations and traits (Breseghello and Sorrells 2007; Gegas et al. 2010), while Tsilo et al. (2010) indicated that the genotypic effects were larger than the environmental effects and $G \times E$ interactions on kernel size and shape.

Large kernel cultivars are preferred by farmers in China because of the belief that large grain is indicative of high yield and quality. Kernel improvement is, therefore, an important breeding objective. The major Chinese wheat areas are usually affected by drought and hot wind stress during the grain-filling period (He and Yu 2008), and these factors seriously affect kernel weight and morphology. It is thus difficult to select superior genotypes for grain characteristics by conventional phenotypic selection, and hence it is necessary to identify important genes for kernel morphology traits and to understand their genetic effects relative to environmental influences.

Molecular markers and statistical analysis programs have enabled cereal geneticists to study complex traits like kernel morphology through genetic maps (Manly et al. 2001; Tsilo et al. 2010) and QTL analysis (Li et al. 2007, 2008a). Various studies of QTL for kernel size and shape indicate inconsistent QTL detection across different experiments, environments and populations (Campbell et al. 1999; Ammiraju et al. 2001; Dholakia et al. 2003; Sun et al. 2009). In the mapping population NY6432-18 × Clark’s Cream, Campbell et al. (1999) detected 40 QTL for kernel size and shape on homeologous groups 1, 2 and 3. In the RIL population derived from PH132 × WL 711, Dholakia et al. (2003) identified 24 QTL for kernel weight, length, width, and a form-density factor on chromosomes 2BL, 2DL, 5BL, 6BS and 7BL. Breseghello and Sorrells (2007) found main kernel morphology QTL on chromosomes 1B, 2D and 5B in the spring wheat crosses W7984 × Opata 85 and AC Reed × Grandin. Recently, stable QTL for kernel size and shape were identified on chromosomes 1A, 2A, 2D, 3A, 4B, 5A, 5B, 6A and 7A (Gegas et al. 2010; Tsilo et al. 2010). In addition, genes GS3, GW2, GIF1 and SW3 for kernel weight and morphology traits in rice were cloned (Song et al. 2007; Wang et al. 2008; Shomura et al. 2008). Sequence-based comparative maps between the rice and wheat genomes have provided opportunities to detect co-localisation of genetic loci between the two species, as discussed by Sorrells et al. (2003). Recently, the orthologs of GW2 and GIF1 were cloned in bread wheat and located on chromosomes 6A and 2A, respectively (Ma et al. 2010; Su et al. 2011).

A 1BL.1RS translocation has been used widely in wheat breeding programs worldwide (Rajaram et al. 1990), particularly in China, where about one-half of the cultivars grown in the primary wheat regions possess this translocation (Zhou et al. 2007). The effect of 1BL.1RS on yield components depends on genetic background and cultural environment, but it generally enhances kernel weight in common wheat backgrounds and different environments (Lelley et al. 2004). Although the effects of chromosome arm 1RS on agronomic traits have been investigated extensively (Graybosch 2001), no study has examined the effect of 1RS on different kernel morphology traits. The studies on mapping kernel morphology traits mentioned above focussed mainly on additive effects, with less attention being paid to epistatic effects, and even less to pleiotropic effects, of QTL among kernel morphology traits. In the present study, a RIL population from a non-1BL.1RS × 1BL.1RS wheat cross, PH × NX (Zhang et al. 2009a, 2009b), was used to identify QTL for kernel morphology traits. Our ultimate objectives were to determine the influence of the 1BL.1RS translocation on kernel morphology traits, and to identify the main-effect and digenic epistatic QTL for these traits and thus achieve a better understanding of the genetic control of the main components of kernel morphology traits and their closely linked molecular markers.

Materials and methods

Plant materials

A mapping population with 240 RIL was obtained from a cross between the non-1BL.1RS cultivar PH and a 1BL.1RS translocation cultivar NX by the single-seed-descent method (Zhang et al. 2009a, 2009b) and used for QTL mapping of kernel morphology traits. PH is a hard white winter wheat cultivar, selected from Fengchan 1/Xiaoyan 55–6/St 2422/464, released in Shandong province in 1992, and NX is a soft white winter wheat cultivar derived from Mianyang 84–27/Neixiang 82c6/Yumai 17, released in Henan province in 2003. Compared with NX, PH is taller and has a larger grain size and higher kernel weight.

Field trials

The field trials were described in detail in Zhang et al. (2009a, 2009b). In brief, a Latinised α-lattice design (Barreto et al. 1997; Zhang et al. 2006) was used in three locations, viz. Anyang (lat. 36°5′13″N, long.114°29′52″E, alt. 67 m) and Jiaozuo (lat. 35°15′42″N, long. 113°15′55″E, alt. 685 m) in Henan Province, and Taian (lat. 36°11′5″N, long. 117°10′58″E, alt. 153 m) in Shandong, in 2006 and 2007, providing data for six environments. The 390 plots were arranged in 13 rows and 30 columns for the 240 RIL, with each plot being six rows, 3 m long and 1.5 m wide, in a seeding rate of 195 seeds m$^{-2}$. Sixty RIL selected randomly were planted with three replicates, whereas the other 180 RIL were arranged as a single replicate. The two parents were included in every column as controls with 15 replicates of each to estimate the field variation. Fungicides and insecticides
were applied at 15-day intervals from heading to mid-grain filling to prevent prevalent diseases and aphid damage.

**Phenotypic data assay**

TKW was measured as the average weight of two independent samples of 500 kernels by an EK-i Portable Precision Balance (Accuweigh, Sydney, Australia) (Zhang et al. 2009b). Twenty well developed kernels from each line were used to measure kernel morphology traits. The ventral side of randomly selected kernels was laid on black corrugated cardboard with five corrugated columns and four rows, and the kernel horizontal direction was random. The kernels were photographed using an Olympus colour camera (Olympus E-500, Shenzhen, China). Each digital picture was 1280 × 960 pixels in size, with 1 mm on the picture being equivalent to 18.1 pixels. Images were analysed using Matlab 6.5 Software (MathWorks, Inc. USA, www.mathworks.com, accessed 25 August 2011) based on the discrete outlines of the kernels in the image to measure the horizontal length, width, perimeter and area. KLEN and KWID were calculated as the major and minor axis lengths of an ellipse, respectively. KP and KA were measured as the perimeter and area, respectively, of the horizontal image. The other traits were determined from TKW (Berman et al. 1996; Bresegello and Sorrells 2007), KLEN, KWID, KP and KA, i.e. SF = 4π × KA/KP², FFD = TKW/(1000 × KLEN × KWID) and KW/L = KWID/KLEN × 100.

**Molecular marker characterisation and linkage map construction**

DNA was extracted from young leaves of the RIL and two parents using the cetyltrimethylammonium bromide method (Sambrook et al. 1989). Protein extractions and separations were performed according to Singh et al. (1991). In total, 2033 simple sequence repeat (SSR) markers, publicly available in GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml; Zhang et al. 2009a, 2009b), one rye secalin marker Sec-I (Francis et al. 1995), the STS marker YP7A for the phytoene synthase gene (He et al. 2008), four glutenin subunit markers (Bx7, Dx5, Glu-B3j, and Glu-A3a), and a CAP marker for the Pinb-D1b allele, were assayed for polymorphisms between the parents. Finally, 195 polymorphic markers were used to genotype the entire population and construct linkage maps for subsequent QTL analysis.

Genetic maps of individual chromosomes were constructed with Map Manager QTX b20 (Manly et al. 2001) using the Kosambi mapping function and P-value 1e⁻⁵. Markers were selected to minimise missing data (q > 40), to avoid segregation distortion (P > 0.01, based on χ²) and to give even coverage of the chromosomes. Linkage groups were assigned to chromosomes based on microsatellite consensus maps (Shi et al. 2003; Somers et al. 2004). The map covered a total length of 2052.5 cM with an average marker interval of 12.4 cM (Zhang et al. 2009a, 2009b). The secalin marker Sec-I/GluB3j indicated the presence of the 1BL/1RS translocation chromosome.

The main-effect QTL and digenic epistatic QTL were identified using ICIM (Li et al. 2007, 2008a), implemented by Windows QTL IciMapping version 2.2 (www.isbreeding.net/download_software_ICIM.aspx). The main-effect QTL analysis was done with the ICIM-ADD model, a scanning step size of 1 cM, the largest P-value for entering variables of 0.01, and the smallest P-value for removing variables of 0.02. The epistatic QTL analysis was performed using the ICIM-EPI model, with a scanning step size of 2 cM, the largest P-value for entering variable of 0.001, and the smallest P-value for removing variable of 0.002. Tail selection was used for null alleles. Parameters for permutation tests were estimated with the ICIM-ADD and ICIM-EPI models, and the values of permutation times and type I errors were 1000 and 0.05, respectively. Main-effect QTL and epistatic QTL were claimed to be significant at a LOD (log₁₀ likelihood ratio) threshold of 2.5.

**Statistical analyses**

Phenotypic data were analysed separately by fitting an appropriate spatial model with controls as a fixed effect and rows, columns, and unreplicated entries as random effects (Gilmour et al. 1997; Zhang et al. 2009a, 2009b). The best-fit model producing the best linear unbiased predictors for the phenotypic data of each genotype in each environment was used in subsequent analyses. Sixty randomly selected lines with three replicates were used for ANOVA and estimates of $h^2$, calculated by the formula $h^2 = \sigma^2_G/\sigma^2_G + \sigma^2_E + \sigma^2_e$, where $\sigma^2_G$ was the genotypic variance, $\sigma^2_E$ was the G × E variance, and $\sigma^2_e$ was the environmental variance.

Statistical analyses were carried out with Proc GLM in SAS software (SAS Institute 2001), with genotypes as a fixed effect, and locations, years, and their related interactions and replicates as random. Pearson correlation coefficients among genotypic means were obtained by means of the CORR procedure of SAS.

**Results**

ANOV A and distribution of kernel morphology traits

All seven kernel morphology traits showed normal distributions, indicative of polygenic characteristics (Fig. 1). Although the parents did not show appreciable differences in these traits, the large ranges of phenotypic variation in the RIL indicated the presence of differing alleles at many loci in the parents for each trait. The $h^2$ for KLEN, KWID, KP, KA, SF, FFD and KW/L, were 0.89, 0.80, 0.84, 0.69, 0.85, 0.61 and 0.88, respectively (Table 1).

The F-values of ANOVA for years, genotypes, environments and G × E interactions are presented in Table 2. There were significant differences among genotypes for all seven traits. The environment also had a large influence on all the seven traits. Significant G × E interactions were observed for all of these traits except SF.

Correlations among kernel morphology traits

TKW was significantly positively correlated with KWID ($r = 0.64, P < 0.001$), KP ($r = 0.53, P < 0.001$), KA ($r = 0.62, P < 0.001$) and FFD ($r = 0.79, P < 0.001$) (Table 3). KLEN was significantly positively correlated with KP ($r = 0.91, P < 0.001$) and KA ($r = 0.68, P < 0.001$). KWID showed a highly significant positive correlation with KP ($r = 0.68, P < 0.001$), KA ($r = 0.87, P < 0.001$) and KW/L ($r = 0.78, P < 0.001$), but was significantly negatively correlated with SF ($r = -0.62, P < 0.001$). A significantly positive correction was identified between KP and KA ($r = 0.85, P < 0.001$), whereas SF and KW/L were negatively correlated ($r = -0.74, P < 0.001$).
QTL analysis
For all seven kernel morphology traits, 71 main-effect QTL were identified across environments in two cropping seasons, with a range of 7–13 QTL for each individual trait (Table 4, Fig. 2). QTL frequency was the highest in the B genome with 37 QTL (52.1%); the other 25 (35.2%) and 9 (12.7%) QTL were found in genomes A and D, respectively. No QTL for kernel morphology traits were found on chromosomes 2B, 4D and 7D. Chromosomes 3D and
Table 1. Summary of ranges and heritabilities ($h^2$) of kernel length (KLEN), kernel width (KWID), kernel perimeter (KP), kernel area (KA), shape factor (SF), factor form-density (FFD), and kernel width/length ratio (KW/L) in the PH 82-2 × Neixiang 188 recombinant inbred line (RIL) population evaluated across six environments in 2006 and 2007

<table>
<thead>
<tr>
<th>Trait</th>
<th>Parent</th>
<th>RIL population</th>
<th>Mean</th>
<th>s.d.</th>
<th>Range</th>
<th>$h^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLEN (mm)</td>
<td>PH 82-2</td>
<td>Neixiang 188</td>
<td>6.7</td>
<td>6.6</td>
<td>5.8–7.4</td>
<td>0.89</td>
</tr>
<tr>
<td>KWID (mm)</td>
<td></td>
<td></td>
<td>3.0</td>
<td>3.1</td>
<td>2.4–3.6</td>
<td>0.80</td>
</tr>
<tr>
<td>KP (mm)</td>
<td></td>
<td></td>
<td>14.5</td>
<td>14.4</td>
<td>12.8–16.1</td>
<td>0.84</td>
</tr>
<tr>
<td>KA (mm²)</td>
<td></td>
<td></td>
<td>292.5</td>
<td>290.1</td>
<td>220.4–362.4</td>
<td>0.69</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.1</td>
<td>0.9–1.2</td>
<td>0.85</td>
</tr>
<tr>
<td>FFD (mg mm⁻²)</td>
<td>0.14</td>
<td></td>
<td>0.1</td>
<td>0.01</td>
<td>0.1–0.2</td>
<td>0.61</td>
</tr>
<tr>
<td>KW/L (%)</td>
<td>48.8</td>
<td>47.0</td>
<td>47.5</td>
<td>2.53</td>
<td>37.1–56.9</td>
<td>0.88</td>
</tr>
</tbody>
</table>

$\text{~}^A$s.d., Standard deviation.

Table 2. ANOVA for kernel length (KLEN), kernel width (KWID), kernel perimeter (KP), kernel area (KA), shape factor (SF), factor form-density (FFD), and kernel width/length ratio (KW/L) in the PH 82-2 × Neixiang 188 recombinant inbred line population evaluated across six environments in 2006 and 2007

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>KLEN</th>
<th>KWID</th>
<th>KP</th>
<th>KA</th>
<th>SF</th>
<th>FFD</th>
<th>KW/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1</td>
<td>0.06</td>
<td>2.86***</td>
<td>4.29***</td>
<td>45715.5***</td>
<td>0.12***</td>
<td>0.00029***</td>
<td>596.7***</td>
</tr>
<tr>
<td>Environment</td>
<td>5</td>
<td>2.98***</td>
<td>2.48***</td>
<td>19.72***</td>
<td>47269.8***</td>
<td>0.05***</td>
<td>0.01065***</td>
<td>241.8***</td>
</tr>
<tr>
<td>Repeatability$^A$</td>
<td>2</td>
<td>0.08***</td>
<td>0.03*</td>
<td>0.37**</td>
<td>404.2*</td>
<td>0.02***</td>
<td>0.00006***</td>
<td>9.7***</td>
</tr>
<tr>
<td>Genotype</td>
<td>59</td>
<td>0.43***</td>
<td>0.12***</td>
<td>1.49***</td>
<td>1121.9*</td>
<td>0.01***</td>
<td>0.00062***</td>
<td>41.2*</td>
</tr>
<tr>
<td>Genotype × environment</td>
<td>295</td>
<td>0.03***</td>
<td>0.02***</td>
<td>0.17***</td>
<td>259.5***</td>
<td>0.001</td>
<td>0.00017***</td>
<td>3.6***</td>
</tr>
<tr>
<td>Error</td>
<td>713</td>
<td>0.02</td>
<td>0.01</td>
<td>0.08</td>
<td>115.3</td>
<td>0.001</td>
<td>0.00003</td>
<td>2.3</td>
</tr>
</tbody>
</table>

$^A$Calculated from 60 lines planted in three replicates.

Table 3. Pearson’s correlations among thousand kernel weight (TKW), kernel length (KLEN), kernel width (KWID), kernel perimeter (KP), kernel area (KA), shape factor (SF), factor form-density (FFD), and kernel width/length ratio (KW/L) in the PH 82-2 × Neixiang 188 recombinant inbred line population evaluated across six environments in 2006 and 2007

<table>
<thead>
<tr>
<th></th>
<th>TKW*</th>
<th>KLEN</th>
<th>KWID</th>
<th>KP</th>
<th>KA</th>
<th>SF</th>
<th>FFD</th>
<th>KW/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLEN</td>
<td>0.37***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>KWID</td>
<td>0.64***</td>
<td>0.40***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>KP</td>
<td>0.53***</td>
<td>0.91***</td>
<td>0.68***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>KA</td>
<td>0.62***</td>
<td>0.68***</td>
<td>0.87***</td>
<td>0.85***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SF</td>
<td>-0.45***</td>
<td>0.14</td>
<td>-0.62***</td>
<td>-0.06</td>
<td>-0.42***</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FFD</td>
<td>0.79***</td>
<td>-0.07</td>
<td>0.12</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.24***</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>KW/L</td>
<td>0.41***</td>
<td>-0.27***</td>
<td>0.78***</td>
<td>0.09</td>
<td>0.45***</td>
<td>-0.74***</td>
<td>0.18</td>
<td>–</td>
</tr>
</tbody>
</table>

$^*P<0.001$; $**P<0.01$; $***P<0.001$

$^A$The data of kernel thousand weight were reported by Zhang et al. (2009b).

QTL for KLEN, KWID, KP and KA

Twelve, 13 and 8 QTL were detected for KLEN, KWID, KP and KA, respectively (Table 4, Fig. 2). Four loci for KLEN on chromosomes 1B, 2A, 4B and 5A were stably identified in more than four environments across two seasons, explaining 3.3–9.2% of the PV. Five stable QTL for KWID on chromosomes 1A, 1B, 2A, 6D and 7A were identified in more than four environments across two seasons, explaining 3.3–19.7% of the PV (Table 4, Fig. 2). Six stable QTL were detected for KP on chromosomes 1RS, 1BL, 2A, 5A, 5B and 6B across two seasons, and the PVE ranged from 2.6 to 18.9% (Table 4, Fig. 2). One table locus for KA, $QKa.caas-1B.1$ linked to Sec-1, was identified in six environments during two cropping seasons, and explained up to 28.2% of the PV (Table 4, Fig. 2).

QTL for SF, FFD and KW/L

Twelve, 7 and 11 QTL were identified for SF, FFD and KW/L, respectively (Table 4, Fig. 2). Four stable loci for SF were detected on chromosomes 1D, 2A, 4B and 7A in more than three environments during two cropping seasons, accounting for 3.5–15.0% of the PV (Table 4, Fig. 2). One stable QTL for FFD located on chromosome 4A was detected in Tai'an in 2006 and 2007, explaining 4.6 and 5.3% of the PV, respectively (Table 4). Six stable QTL for KW/L were identified on chromosomes 1A, 2A, 1D, 5B, 6B and 7A in more than three seasons.
Table 4. Quantitative trait loci (QTL) for kernel length (KLEN), kernel width (KWID), kernel perimeter (KP), kernel area (KA), shape factor (SF), factor form-density (FFD), and kernel width/length ratio (KW/L) identified in the PH 82-2 x Neixiang 188 recombinant inbred line population evaluated across six environments in 2006 and 2007.

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</thead>
<tbody>
<tr>
<td>KLEN</td>
<td>QKlen.caas-1B.1</td>
<td>Sec-1-HVM23</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QKlen.caas-1B.2</td>
<td>Glu-B1-Xbarc61</td>
<td>7.3</td>
<td>7.7</td>
<td>8.9</td>
<td>9.2</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QKlen.caas-1D</td>
<td>Xbarc169-Glu-D1</td>
<td>5.0</td>
<td>5.1</td>
<td>5.9</td>
<td>–</td>
<td>–</td>
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(continued next page)
environments across two seasons, and the PVE for the QTL ranged from 3.4 to 9.9% (Table 4, Fig. 2).

**Co-locations among the QTL for kernel morphology traits**

Eight QTL clusters for TKW and seven kernel morphology traits were detected in the RIL population (Fig. 2). Four QTL for KLEN, KWID, KP and KA in the Sec-1-HVM23 interval on chromosome 1BL.1RS were related to the TKW QTL (Zhang *et al.* 2009b; Fig. 2), and the favourable allele was from NX with the 1BL.1RS translocation. Similarly, other QTL clusters located on chromosomes 1BL, 2A, 4A, 4B, 6B, 6D and 7A for TKW (LOD = 2.8–2.9, not shown in Zhang *et al.* 2009b) and kernel morphology traits, viz. KLEN, KWID, KP, KA, SF, FFD and KW/L, were co-located in approximately the same place. These co-located loci were consistent with the significant correlation coefficients (*r*) (Table 3).

**Epistatic QTL for kernel morphology traits**

Fourteen pairs of epistatic QTL among 24 loci showed significant effects on all traits except KW/L (Tables 4 and 5). One epistatic QTL for KLEN was found between two loci on chromosome 5B, accounting for 2.7% of the PV. Two epistatic QTL for KWID were detected between QKwid.caas-3B and QKwid.caas-6D and between QKwid.caas-6B and QKwid.caas-6D, explaining 8.3 and 5.3% of the PV, respectively. Two pairs of epistatic QTL were identified for each of KP and KA, accounting for 2.5–5.0% of the PVE (Table 5). There were four and three pairs of epistatic QTL for SF and FFD, respectively. Each of epistatic QTL for SF and FFD explained 3.2–6.8% and 2.8–3.0% of the PV, respectively (Table 5).

**Discussion**

**Pleiotropic effects of QTL for kernel morphology traits**

Pleiotropic effects of genes for different traits are often reported in wheat (Campbell *et al.* 1999; Sun *et al.* 2009; Zhang *et al.* 2009a, 2009b; Tsilo *et al.* 2010). In the present study, alleles contributed by NX at the locus Sec-1 on 1RS conferred increases in KLEN, KWID, KP and KA, consistent with the significantly positive correlations among them. Bergman *et al.* (2000) and Bresegello and Sorrells (2007) also reported pleiotropic effects on variation in kernel morphology traits, although the traits were inherited independently. The QTL for kernel morphology traits on 1RS was not reported previously, and 1RS also had large influences on kernel weight, flour colour, noodle colour, mixograph parameters (Zhang *et al.* 2009a, 2009b) and glutenin protein fractions (Zhang *et al.* 2011) in the same population.

Another important locus for KLEN, KP, KA and FFD on chromosome 1BL was located near the Glu-B1 region, in agreement with the previous reports (Campbell *et al.* 1999; Sun *et al.* 2009). The loci controlling kernel morphology traits had pleiotropic effects on noodle quality, mixograph, RVA (rapid viscosity analyser) parameters (Zhang *et al.* 2009a, 2009b) and quantities of glutenin protein fractions (Zhang *et al.* 2011). Some QTL for kernel morphology traits had pleiotropic effects on grain quality (Bresegello and Sorrells 2007). It seems that similar functions are conferred by multiple linked QTL positioned in specific regions, or by a single gene with multiple functions. In maize, QTL affecting kernel weight were located near a locus encoding starch synthesis enzymes (Berke and Rocheford 1995). Several linkage groups carrying QTL for kernel morphology were also associated with β-glucan and oil concentration in two hexaploid oat populations (Groh *et al.* 2001).

A new locus for KLEN, KP, SF and KW/L linked to marker Xcfe53 was detected near the centromere of chromosome 2AL in more than four environments during the two cropping seasons. Other studies also identified QTL for test weight, TKW, KWID and SF, on the distal region of the chromosome 2AL (Campbell *et al.* 1999; Sun *et al.* 2009). Recently, Ma *et al.* (2010) cloned TaCwi-A1, encoding a critical enzyme for sink tissue development and carbon partition, and it was significantly associated with kernel weight and located on chromosome 2AL. Thus in various studies of QTL in wheat and barley

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**Table 4.** (continued)

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^ The percentage of the variance explained by the QTL.
Fig. 2. Chromosomal locations of quantitative trait loci (QTL) for kernel length, kernel width, kernel perimeter, kernel area, shape factor, factor form-density, and kernel width/length ratio mapped in the PH 82-2 × Neixiang 188 recombinant inbred line population. Putative QTL confidence intervals with a LOD (log₁₀ likelihood ratio) score ~2.5 are shown by vertical bars, whereas LOD max is indicated by a black horizontal line. When genetic distances exceed 50 cM between two markers in the current map, the gaps are indicated with hatched lines. Kernel thousand weight QTL on 1BL.1RS, 4A, 7A with LOD scores ~3.0 were reported by Zhang et al. (2009b), but additional QTL on 1BL, 2A, 4B, 6B and 6D with LOD scores ~2.5 and <3.0 were not reported in that study. The symbols (1) to (8) were QTL clusters for the multiple genes on single chromosomes.
Fig. 2. (continued)
(Ayoub et al. 2002; Breseghello and Sorrells 2007), genes on chromosomes 2D and 2H conferred major effects on kernel size and shape. Yoshida et al. (2002) also reported a major QTL for rice kernel shape and milling efficiency on chromosome 4, which showed maximum homology to wheat group 2 chromosomes (Sorrells et al. 2003).

Besides the stable QTL clusters on chromosomes 1RS, 1BL and 2AL, some major QTL co-mapped on chromosomes 4A, 4B, 6B, 6D and 7A. Allelic variation of the orthologous GS3 gene in common wheat was detected on chromosome 4AL in a RIL population derived from the cross W7984 × Opata85, and the locus accounted for 19.0% of PV for kernel weight (Li et al. 2008b). Gegas et al. (2010) reported a major QTL for grain weight and width near Rht-B1 allele on chromosome 4BS, whereas the QTL for KLEN, KP and FFD co-localised with TKW and test weight were located near the centromere of chromosome 4BL.
A QTL cluster for KWID, KP, KA, TKW and RVA was identified on chromosome 6D in the population (Table 4; Zhang et al. 2009b), in agreement with the results of Giura and Saulescu (1996). The orthologue GW2 on chromosome 6A in common wheat was significantly associated with KWID and weight (Su et al. 2011) and presence of

Table 5. Digenic epistatic quantitative trait loci (QTL) for kernel length (KLEN), kernel width (KWID), kernel perimeter (KP), kernel area (KA), shape factor (SF), factor form-density (FFD), and kernel width/length ratio (KW/L) identified in the PH 82-2 × Neixiang 188 recombinant inbred line population evaluated across six environments in 2006 and 2007

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A Add-Add is the effect of additive-by-additive interaction between two QTL.
B Phenotypic variance explained by interaction of two QTL.
C LOD, log10 likelihood ratio.
a particular allele increased average TKW by more than 3 g in a test of 265 Chinese wheat cultivars (Su et al. 2011). A QTL cluster for KW, SF, FFD and KW/L near the centromere of chromosome 7A was co-located in the same marker interval as QTKw.caas-7A4 and QRFv.caas-7A (Zhang et al. 2009b), in agreement with Kumar et al. (2006) who located QGw.caas-7A1 for grain weight in a RIL population. Several other studies also identified QTL for TKW and test weight near the centromere of chromosome 7A (Campbell et al. 1999; Ammiraju et al. 2001; Groos et al. 2003; Huang et al. 2004).

In summary, we detected stable QTL clusters influencing TKW and kernel morphology traits on chromosomes 1RS, 1BL, 2AL, 4B and 7A. Molecular markers closely linked to these clusters, viz. Sec-I on 1RS for TKW, KWD, KP and KW/L, Glu-Bl on 1BL for TKW, KLEN and KP, Xcfe53 on 2A for KLEN, KP, SF and KW/L, Xwmc238 on 4B for KLEN, KP, SF, FFD and KW/L, and Xbarc174 on 7A for TKW, KWD, KP, SF and KW/L could be used in wheat breeding to improve kernel weight and size uniformity.

Associations between kernel morphology traits and TKW

The efficiency of indirect selection depends on the correlation between a selected trait and a target trait as well as the heritability of the selected trait. Gegas et al. (2010) confirmed that kernel size and shape were largely independent traits in a study of six wheat populations. Like TKW, as reported by Zhang et al. (2009b), most kernel morphology traits showed high heritability ($h^2 > 0.80$) except for KA and FFD ($h^2 < 0.80$, Table 1). TKW exhibited superior correlation coefficients with FFD ($r = 0.79$), moderate with KWD, KP and KA ($r > 0.50$), and minor with KLEN, SF and KW/L ($r < 0.50$). Based on the QTL clusters identified in this study (Fig. 2), eight loci for TKW on chromosomes 1RS, 1BL, 4A, 4B, 6D and 7A were also associated with KLEN, KWD, KP, KA, SF, FFD and KW/L. The genetic correlations estimated here were consistent with previous observations in winter wheat (Sun et al. 2009), barley (Ayyoub et al. 2002), and sunflower (Yue et al. 2008). The results showed that the genotypic correlations among these traits were caused by closely linked genes or genes with pleiotropic effects (Xu 1997).

Influence of 1BL.1RS translocation on kernel morphology traits and TKW

The 1RS chromosome arm benefits yield potential, particularly kernel weight (Lelley et al. 2004). Other than the study of Schlegel and Meinel (1994), who postulated the presence of a QTL on chromosome 1BL.1RS for spikelet fertility, no studies have been conducted on QTL mapping for yield and yield components. In the present study, QTL for KLEN, KWD, KP and KA were identified on the short arm of chromosome 1BL.1RS with increasing effects from NX alleles, and the loci coincided with a TKW QTL in the same population as mentioned above (Zhang et al. 2009b). Thus many genes were associated marker Sec-I on 1RS. This association could be caused by any of three factors: (i) 1RS should be a non-recombining linkage block in this cross, (ii) the region near the secalin locus contains certain genes controlling an array of important grain traits besides quality characters (Erayman et al. 2004), and (iii) in some genetic backgrounds, the 1BL.1RS translocation increases drought tolerance and adaptability (Graybosch 2001), making such genotypes more propitious in terms of root biomass, water-use efficiency, transpiration, and dry matter accumulation (Ehdai et al. 2003), delayed maturity, and an extended grain-filling stage (Villarel et al. 1998). Consequently, 1RS in 1BL.1RS can have a large influence on kernel morphology traits and TKW.

Epistatic effects influence kernel morphology traits

The heritable genetic components include main-effect and epistatic effects of genes. These two components were statistically separated by two loci analysis (Table 5). Among the seven kernel morphology traits, only KW/L was not influenced by epistatic interaction. However, the total PVE by main-effect QTL ranging from 8.1% for SF to 54.1% for KP across environments was much higher than the total PV (ranging from 2.7% for KLEN to 18.8% for SF) contributed by epistatic QTL. This indicated that the genetic contribution of main-effect QTL for kernel morphology traits were greater than epistatic effects, in agreement with Ammiraju et al. (2001). However, Novoselovic et al. (2004) found additive-by-additive epistasis was more important than additive effects for grain weight and other yield components in two winter wheat crosses by generation mean analyses. In rice, epistatic QTL explained a larger proportion of the phenotypic variation than main QTL for grain weight and grain width (Li et al. 1997; Lou et al. 2009). In Arabidopsis thaliana, the magnitude of epistatic effects was roughly double the effects of additive QTL for seed length and width (Malmborg et al. 2005). Although some loci were identified as additive and also involved in epistatic interactions in the present study, epistatic interactions played an indispensably significant role, especially for SF and FFD. The genetic architecture of kernel morphology traits is a network of additive and epistatic effects; therefore, to select a cultivar with excellent kernel morphology traits, breeders need to consider all genes enhancing kernel morphology traits, and to make crosses that are sufficiently wide to remove alleles capable of conferring negative effects.

Acknowledgements

The authors are grateful for the critical review of this manuscript by Professor R. A. McIntosh, Plant Breeding Institute, University of Sydney. This study was supported by the National Basic Research Program (2009CB118300), the National Science Foundation of China (31161140346), an international collaboration project from the Ministry of Agriculture (2006-G2), and an earmarked fund for the Modern Agro-industry Technology Research System.

References


