Inheritance and QTL analysis of flour falling number using recombinant inbred lines derived from strong gluten wheat ‘Gaocheng 8901’ and waxy wheat ‘Nuomai 1’

Zhijing Deng¹, Fang Chen², Shuna Hu¹, Qingdian Han², Jiansheng Chen¹, Cailing Sun¹, Yongxiang Zhang¹, Shouyi Wang¹, Xuejiao Song¹, Jichun Tian²

¹State Key Laboratory of Crop Biology, Key Laboratory of Crop Biology of Shandong Province, Group of Wheat Quality Breeding, Shandong Agricultural University, Taian, Shandong, P.R. China, 271018
²College of life science, Linyi University, Linyi, Shandong, P.R. China, 276005

Abstract

Flour falling number (FN) is an important trait because not only related with pre-harvest sprouting (PHS) tolerance but also with processing quality. Waxy wheat usually has low flour falling number (FN) without severe pre-harvest sprouting (PHS). To identify quantitative trait loci (QTLs) associated with flour FN, a recombinant inbred line (RIL) population derived from two Chinese wheat, strong gluten wheat ‘Gaocheng 8901’ and waxy wheat ‘Nuomai 1’, was employed to evaluate the genetic variation and to detect QTLs based on DAR-T and SSR markers under three environments. It was shown that the higher variation existed in the RIL for FN. Ten additive QTLs were identified, of which four QTLs (QFN.sdau-4A.1, QFN.sdau-4A.2, QFN.sdau-7A and QFN.sdau-7D2.1) located on chromosome 4A, 7A and 7D, respectively, showed stable in different environments around the Wx genes loci for FN. In total, twenty three epistatic QTLs were screened, in which three epistatic QTLs (QFN.sdau-4A.1/ QFN.sdau-7A, QFN.sdau-4A.1/QFN.sdau-7D2.1 and QFN.sdau-7A/QFN.sdau-7D2.1) showed stable in different environments. Significant effects were found in almost all QTLs detected. We could use the molecular markers linked with the Wx loci to accelerate the development of cultivars with high flour FN and low PHS in marker-assisted selection.

Keywords: Chinese wheat; flour falling number; additive QTLs; epistatic QTLs.

Abbreviations: FN-falling number; GBSSI-granule-bound starch synthase I; GSL-general superior lines; MAS-marker-assisted selection; PHS-pre-harvest sprouting; PVE-phenotypic variation explanation; QTLs-quantitative trait loci; RIL-recombinant inbred line; SL-superior line.

Introduction

Hagberg falling number (FN) is an important trait in wheat. This method was developed by Hagberg (1961) and Perten (1964) in the early 1960s. Due to its simplicity, rapidness, and reliability, it has become the standard technique for determining α-amylase activity using wheat meal, and accepted by the International Association of Cereal Science and Technology (ICC, 1968), followed by the American Association of Cereal Chemists (AACC, 1972). Because the high flour FN is a very important parameter to determine the processing of flour quality, it has been widely used in grain classification and quality control in the wheat industry.

Sprout damage is often assessed and quantified by the Hagberg flour FN test, which allows determination of starch degradation resulted from early activation of α-amylase prior to any visible sprouting symptoms. Several early studies have suggested that PHS resistance may not be necessarily associated with the high flour FN. Nevertheless, flour FN is a very important parameter to characterize the PHS damage in wheat (Finney, 1985).

In general, flour FN is negatively associated with α-amylase activity. Cultivars with a low flour FN will not only show yield reduction, but also the quality of processing, storage and end-product due to high α-amylase activity (Edwards et al., 1989). As a result of such precocious and permanent levels of α-amylase expression, cultivars with low flour FN often show pre-harvest sprouting (PHS) (Mares and Mrva, 2008). However, such inverse relationship between flour FN and α-amylase activity needs to be carefully evaluated since the flour FN trait is affected by both enzyme and substrate, and these products are the results of interactions between genotypes and environments, under which the grain samples develop and become mature. Graybosch et al. (2000) demonstrated that a waxy wheat with very low flour FN generated much reduced amylose content, the substrate of α-amylase, compared to a normal genotype, suggesting genotypic variation should be taken into consideration, and low flour FN does not necessarily always lead to high level of α-amylase followed by PHS.

The rapid developments of molecular marker technologies and QTL analyses have allowed us to identify and evaluate the effects of QTL for PHS and flour FN traits more effectively and accurately. Recently, several laboratories have conducted studies on QTLs for PHS and its related traits such as seed dormancy, α-amylase activity, grain color, seed germination in bread wheat (Kulwal et al., 2004; Law et al., 2005; Mohan et al., 2009; Mrva et al., 2009; Rasul et al., 2009; Emebiri et al., 2010; Sing et al., 2012), barley (Emebiri et al., 2004 ), and rye (Masoj and Milczarski, 2009; Tenhola-Roininen et al., 2011). Several important QTLs for PHS in wheat have been detected on chromosomes 3A, 3B, 3D, 4A, 4B, 5A, 6A, 6B, 7B and 7D (Kulwal et al., 2004; Law et al., 2005; Mrva et al., 2009; Rasul et al., 2009; Emebiri et al., 2010; Sing et al., 2012). Similarly, using diverse mapping populations, several QTLs for flour FN in wheat have been located on chromosomes 4A, 4B, 3A, 3B, 3D (Law et al., 2005; Kunert et al., 2007; Imtiaz et al., 2008;
Fofana et al., 2009; Rasul et al., 2009). These studies suggest the importance of these chromosomes (4A, 4B, 3A, 3B, 3D) in determining flour FN and PHS in wheat. However, these results were primarily based on the use of the populations that were always associated with PHS.

On the contrary, we developed a novel RIL population derived from crossing ‘Nuomai1’ (a waxy wheat without the three waxy alleles) with ‘Gaocheng8901’ (a strong gluten wheat with the three normal waxy alleles). It would be interesting to investigate the possible molecular mechanism(s) underlying this phenomenon. The uniqueness of such population is that many quality traits, especially flour FN, showed large variations without PHS. To the best of our knowledge, this is the first report on QTLs on flour FN analyses using such unique populations.

Therefore, we attempted to use our novel RIL population to detect the QTLs for flour FN without any PHS effect; thereby, to evaluate the genetic effects and elite genotypes in various environments. Ultimately we aimed (1) to pinpoint the flour FN without PHS at the molecular level and to identify the new QTLs controlling flour FN that are not linked to PHS; (2) to accelerate the development of superior varieties with high flour FN QTLs, but without PHS, using marker-assisted selection in wheat breeding programs.

Results

Phenotypic variation of the RILs and parents

Data listed in Table 1 showed a pattern of continuous variations for almost all flour FN traits examined under each of the three environments. Transgressive segregants (higher than that of the high flour FN parent or lower than that of low flour FN parent) were present in this RIL population, due to the contribution of alleles with positive effects derived from both parents. Clearly, the two parents differed in each of the three environments.

QTLs for flour FN

Of the 1052 markers, 498 (47.3%) were found to be polymorphic in the two parents, and these markers were used for linkage analyses as well as for flour FN QTL analyses. We used these 498 markers to construct a genetic linkage map which included the 479 DARt, 14 SSR, 2 HMW-GS and 3 Wx protein makers. This map covered 4229.7 cM in length with a mean of 9.77 cM. These markers were distributed on 21 chromosomes of the three homoeologous genomes (A, B and D). A total of 24 linkage groups were constructed. The three genomes A, B and D had 211, 166 and 121 markers, respectively.

Additive QTLs

As shown in Table 2, 10 QTLs were detected and mapped on eight chromosomes, involving 1A, 2D, 4A, 1D, 6B, 6D, 7A and 7D. Three QTLs were identified under each individual environment. Based on the data of three years (PD) under three conditions (E1, E2 and E3), nine QTLs were detected, and two of them (QFn.sdau-7A and QFn.sdau-7D2.1) were found in all three environments, implying their stability. While QFn.sdau-4A.1 was detected under the conditions of E1, E3 and PD, QFn.sdau-4A.2 was only detected in E2. Both QFn.sdau-4A.1 and QFn.sdau-4A.2 markers were located nearby the Wx-B1 locus, suggesting these two QTLs might share the same interval.

In addition, of these ten QTLs, two of them (QFn.sdau-2D and QFn.sdau-1D2.1) showed positive additive effects, suggesting these positive alleles for these QTLs were most likely derived from Nuomai1 even though such effects could be detected only based on PD (Table 2). However, negative additive effects were found from the other eight QTLs, suggesting such inverse effect was possibly contributed by Gaocheng8901. The phenotypic variation (PVE) of individual QTL (R²) ranged from 0.25 (QFn.sdau-6B) to 11.92% (QFn.sdau-4A.1) (Table 2). Moreover, no obvious interaction between examined QTLs and environment was found, suggesting that these QTLs are relatively stable and are controlled mainly by additive alleles, which potentially are ideal for marker-assisted breeding.

Epistatic QTLs

A total of 23 pairs of epistatic QTLs were identified, which involved in chromosomes 1A, 1B, 1D, 2A, 2D, 3A, 3B, 3D, 4A, 5A, 5B, 6A, 6B, 6D, 7A and 7D, explaining for 0.09%-10.88% of the observed variations (Supplementary Table 1). All the epistatic effects occurred from the results of interactions between non-linkage loci located on different chromosomes (Fig. 1 and Supplementary Fig. 1).

Interaction between two loci, QFn.sdau-7A and QFn.sdau-7D2.1 were detected in all three environments and PD, with a PVE ranging from 8.07% to 10.88% (Supplementary Table 1). The interactions of two other pairs (QFn.sdau-4A.1 and QFn.sdau-7A), and (QFn.sdau-4A.1 and QFn.sdau-7D2.1) were also identified in E1, E3 and PD, albeit with a lower PVE (Supplementary Table 1). Meanwhile, six loci, QFn.sdau-4A.1, QFn.sdau-4A.2, QFn.sdau-1A1, QFn.sdau-1A2, QFn.sdau-1D and QFn.sdau-6B, showed epistatic effects, resulted from interactions with more than one QTL locus (Supplementary Table 1). Interestingly, no interaction between any epistatic QTLs and any environments could be detected, indicating these epistatic QTLs were stable since environment played little or no role in controlling these loci. Clearly, these epistatic loci are of great significance in marker-assisted selection breeding programs.

Ten additive QTLs and twenty three epistatic QTLs were dealt with sixteen chromosomes. Most importantly, it seems that the additive QTL played more important role than the epistatic for flour FN.

Predicting superior genotype

In order to better utilize the mapped QTLs for improving flour FN traits in bread wheat and ultimately to develop breeding lines carrying desirable QTLs, genotypes with the mapped QTLs of interest were selected. It was done through comparing the total genetic effect of each individual QTL with those known genotypes within the RILs population.

We estimated all of the possible genotypic combinations of QTLs for flour FN based on the additive and epistatic effects and determined those as flour FN general superior lines (GSL) when maximum genetic effects (both additive and epistatic) were reached (Table 3).

In general, a higher flour FN will result in low α-amylase activity. Hence, high flour FN should be chosen for GSL prediction. The estimated total genetic effects for the higher parent (P2, Gaocheng8901) and SLs varied greatly in different environments. The predicted total genetic effects of SLs were far higher than that of P1 in each environment and P2 in E2, E3
Table 1. Flour FN phenotypic data of the two parents and the RIL lines under three environmental conditions.

<table>
<thead>
<tr>
<th>Env</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>255</td>
<td>394.67</td>
<td>133.45</td>
<td>68.50</td>
<td>584.00</td>
<td>-1.41</td>
<td>1.23</td>
</tr>
<tr>
<td>E2</td>
<td>248</td>
<td>411.2</td>
<td>135.65</td>
<td>69.00</td>
<td>599</td>
<td>-1.22</td>
<td>1.02</td>
</tr>
<tr>
<td>E3</td>
<td>245</td>
<td>392.93</td>
<td>135.83</td>
<td>68.00</td>
<td>586.00</td>
<td>-1.36</td>
<td>1.04</td>
</tr>
<tr>
<td>PD</td>
<td>768</td>
<td>399.58</td>
<td>135.04</td>
<td>68</td>
<td>599</td>
<td>-1.32</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Note: E1, E2, and E3 represent the environments of 2008 Taian, 2009 Taian, and 2011 Suzhou, respectively. PD: Data of three years. Abbreviations are: environment (Env), number of samples (N), standard deviation (SD), minimum (Min), maximum (Max).

Fig 1. The genetic architecture of flour FN QTLs generated with QTL Network 2.0. A: QTLs detected in Taian location in the year 2008. B: QTLs detected in Taian location in the year 2009. C: QTLs detected in Suzhou location in the year 2011. Red dot: additive effect; Black dot: no additive effect; Blue dot: additive×environment effect; Red line: Epistetic effect (interaction between two QTLs).
and PD except E1, indicating the large potential for genetic improvement of flour FN and an over parent dominant effect existing.

Data illustrated in Supplementary Table 2 indicated that those QTL GSl genotypes expressed in all three conditions and PD, QQ alleles were contributed by P1 (Nuomai 1), whereas qq alleles were derived from P2 (Gaocheng 8901). Compared with P1, the predicted GSl QTL genotypes differed at fifteen loci, seven of which showed additive effects (QFn.sdau-4A.1; QFn.sdau-7A; QFn.sdau-4A.2; QFn.nset-1A1.1; QFn.sdau-1D.1; QFn.sdau-6B.1; QFn.sdau-6D), and eight of which expressed as non-individual QTLs (QFn.sdau-3D; QFn.sdau-1A1.2; QFn.sdau-1A2; QFn.sdau-1D.4; QFn.sdau-1D.5; QFn.sdau-6A2; QFn.sdau-2A.1; QFn.sdau-3A.2) (Table 5). These QTL alleles are responsible for expressions of high flour FN, which were contributed by P2. These results suggest that the superior QTL alleles present in P2 can replace the inferior ones present in P1 rapidly through marker-assisted selection.

Discussion

It has been generally accepted that flour FN is a very important trait in wheat processing quality due to its association with PHS. Cultivars with low flour FN often result in severe PHS because of their excessive α-amylase activity, leading to yield losses and poor flour quality (Mares et al., 2005; Kottearachchi et al., 2006). Conversely, those cultivars with high flour FN often show no PHS. Clearly, developing varieties with high flour FN parameters are one of pivotal goals in wheat breeding. However, this flour FN and PHS relation should be considered as a general guideline, but not as a rule. Some studies have shown that low flour FN does not necessarily lead to high level of this starch enzyme that will result in PHS (Graybosch et al. 2000). This suggests that it is possible to develop varieties with low flour FN without PHS.

In this study, we found that the flour FN parameters of both parents and the RIL population have a wide range of variations which exhibited a pattern of continuous distribution. These results strongly suggest that flour FN traits belong to quantitative inheritance as reported previously (Kulwal et al., 2004; Law et al., 2005; Mohan et al., 2009; Mrva et al., 2009; Emebiri et al., 2010; Sing et al., 2012). We showed that eight putative QTLs with additive effects for flour FN were contributed by Gaocheng 8901 and were distributed on chromosomes 1A, 1D, 4A, 6B, 6D, 7A and 7D. In agreement with several other studies (e. g., Kunert et al., 2007; Fofana et al., 2009; Emebiri et al., 2010), we could not identify any similar QTLs for flour FN on chromosomes 3B, 4B and 7B. The lack of enough molecular markers mapped on these chromosomes, in particular on their long arms, might be responsible for such failure even though QTLs for PHS on the long arms of these chromosomes had been reported previously.

In this study, several important QTLs for flour FN including two on chromosome 4A and one on each of 7A and 7D were located in close vicinity of the Wx-B1, Wx-A1 and Wx-D1 genes which are responsible for GBSSI activity and are associated with the α-amylase synthesis. The total PVE for flour FN ranged from 22.29% to 31.53%. To our knowledge, this is the first report to reveal the physical neighboring relations between the QTLs for flour FN and the Wx genes. QTLs associated with seed dormancy, sprouting index and germination index, all of which are related to flour FN and PHS have also been mapped on chromosome 4A (Filimham et al., 2002; Mares et al., 2005; Mori et al., 2005; Torada et al., 2005; Raoul et al., 2008) and Gaocheng 8901 (M. Kottearachchi et al., 2006) reported that one QTL (QPhs.o.cs-4A.1) controlling PHS resistance through seed dormancy in a red grain wheat cultivar was located on 4AL, and this gene is homeologous to SD4 on chromosome 4H in barley. Coincidently, we found two QTLs, QFbd.sdau-4A and QFsh.sdau-4A, controlling breakdown and setback (the RVA parameters, data not shown) were within the same marker interval as QFn.sdau-4A.1 (QTL for FN). McCartney et al. (2006) also reported that one QTL, QRpv.crc-4A, for RVA peak viscosity is located on chromosome 4AL. In fact, RVA parameters affect flour FN indirectly through starch enzyme activity to control sprouting. Taken together, these results strongly suggest that the QTLs mapped on chromosome 4AL are most important traits controlling flour FN and PHS.

Contradictory results regarding whether effect of environment would affect flour FN have been reported previously, in which some studies suggested “yes” (Zanetti et., 2000), others indicated “no” (Kulwal et al., 2004; Mohan et al., 2009). Our data were in agreement with the latter since we were not able to detect interactions between QTLs examined and environments. This may be caused by the fact that the parents did not differ for the QTL that interact with the environment, although the presence of such QTL in wheat genome should not be ruled out. In addition to additive QTLs, the role of epistasis QTL in controlling quantitative genetic variation for flour FN in wheat has also been demonstrated. A number of such epistatic QTLs for presented in this report suggest that QxQ interaction is crucial for flour FN.

Materials and Methods

Plant materials

A recombinant inbred line (RIL) population consisting of 290 lines through single seed descent approach (until F8) was developed from a cross between two winter wheat cultivars, namely Nuomai 1 (female) and Gaocheng8901 (male). Nuomai 1 (Jiangsu Baihuomai/Guandong107) carrying HMW-GS, known as alleles of Ax-null, Bx7 + By8, and Dx2.2 + Dy12 at the Glu-A1, Glu-B1, and Glu-D1 loci was developed by China Agricultural University and was released in 2005 in Beijing, China. It owns three null waxy alleles (Wx-A1b, Wx-B1b, and Wx-D1b), similar to red winter wheat. Moreover, this cultivar has unique starch properties that are related to high-quality white salt noodles. Consisting of normal waxy alleles, Gaocheng8901 (77546-2/Lingzhang) was bred by Gaocheng Agricultural Science Research Institute and was released in 1998 in Hebei province, China. This cultivar carries HMW-GS, namely alleles of Ax1, Bx7 + By8, and Dx5 + Dy10 at the Glu-A1, Glu-B1, and Glu-D1 loci. It exhibits high gluten strength and good bread-making quality.

Experimental design

The field trials were conducted over three years utilising a randomised complete block design. Two replicates were conducted at the experimental fields of Shandong Agricultural University, Tai’an City (36°55′N, 116°36′E) in the harvest years 2008 (E1) (10 June) and 2009 (E2) (8 June) and of Szhou (33°38′N, 116°58′E) in the harvest year 2011 (E3) (1 June), Anhui Province, China. For the trial under each site-year, all RIL lines and the two parents were grown in four-row plots with 2 m-long, and 26 cm-wide in-between each two rows, respectively. During the growing season, standard local practices were followed for crop management, and the plants were healthy without obvious damage caused by disease and pests, or symptom of PHS.
Table 2. Additive effects of QTL for flour falling number.

<table>
<thead>
<tr>
<th>Env</th>
<th>QTL</th>
<th>Marker interval</th>
<th>Position(cM)</th>
<th>Range(cM)</th>
<th>A</th>
<th>R² (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>QFn.sdau-4A.1</td>
<td>wPt664948-WX-B1</td>
<td>96.4</td>
<td>90.4-116.8</td>
<td>-40.49</td>
<td>8.07</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QFn.sdau-7A</td>
<td>wPt731311-WX-A1</td>
<td>161.9</td>
<td>152.9-161.9</td>
<td>-34.71</td>
<td>6.18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QFn.sdau-7D2.1</td>
<td>WX-D1-wPt644368</td>
<td>0</td>
<td>0.0-9.0</td>
<td>-42.88</td>
<td>8.04</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22.29</td>
</tr>
<tr>
<td>E2</td>
<td>QFn.sdau-4A.2</td>
<td>WX-B1-wPt0105</td>
<td>108.8</td>
<td>93.4-115.8</td>
<td>-45.57</td>
<td>7.08</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QFn.sdau-7A</td>
<td>wPt731311-WX-A1</td>
<td>161.9</td>
<td>151.9-161.9</td>
<td>-34.38</td>
<td>5.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QFn.sdau-7D2.1</td>
<td>WX-D1-wPt644368</td>
<td>0</td>
<td>0.0-10.0</td>
<td>-49.94</td>
<td>11.6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24.38</td>
</tr>
<tr>
<td>E3</td>
<td>QFn.sdau-4A.1</td>
<td>wPt664948-WX-B1</td>
<td>96.4</td>
<td>90.4-102.8</td>
<td>-41.82</td>
<td>11.92</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QFn.sdau-7A</td>
<td>wPt731311-WX-A1</td>
<td>161.9</td>
<td>152.9-161.9</td>
<td>-34.26</td>
<td>8.62</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>QFn.sdau-7D2.1</td>
<td>WX-D1-wPt644368</td>
<td>0</td>
<td>0.0-9.0</td>
<td>-44.59</td>
<td>10.99</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31.53</td>
</tr>
</tbody>
</table>

For abbreviations of E1, E2, E3 and PD, see the footnotes in Table1. A: additive effect. R²: phenotypic variation explained by individual QTL.

Table 3. Predicted genetic effects (G) for P1, P2 and a superior line (SL) for flour FN.

<table>
<thead>
<tr>
<th>Entry</th>
<th>G</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>-253.029</td>
<td>-262.4059</td>
<td>-254.1508</td>
<td>-344.8371</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>118.0819</td>
<td>129.8955</td>
<td>119.9243</td>
<td>88.9704</td>
<td></td>
</tr>
<tr>
<td>SL(+)</td>
<td>87.7329</td>
<td>70.6986</td>
<td>87.9801</td>
<td>394.1438</td>
<td></td>
</tr>
</tbody>
</table>

For abbreviations of E1, E2, E3 and PD, see the footnotes in Table1. Other abbreviations: G (genetic effects); P1 (parent1); P2 (parent2); SL (superior line).

Mill flour

Seed samples obtained from the harvest populations were normally stored for about one month and then milled using a Bühler experimental mill (Bühler, Bühler-Miag Company, Germany) with a flour extraction yield of approximately 70%.

Flour falling number test

Hagberg flour Falling Number was determined according to method 56-81B (American Association of Cereal Chemists 2000) on a Perten Falling Number 1500 instrument (Perten Instruments Inc., Springfield, IL) applied on wheat flour. A representative sample of 7 g flour (14% moisture content) was placed in a flour FN test tube and 25±0.2 ml of distilled water was added to form the slurry. The tube containing the slurry was shaken until no dry flour was observed at the bottom of the tube before being placed in the machine, then the plungers at 5s started to work. The time required for the plungers to fall from the top to the bottom at 60s of the tube determined the flour FN in seconds. The flour of all samples was weighed on a 14% moisture basis before testing. Results were presented as the flour FN mean of two replicates.

QTL analysis

The molecular genetic map was constructed by using MAPMAKER/EXP ver 3.0b software (Lincoln et al., 1993). A recombination frequency of 0.4 and an LOD value of 3.0 were used as threshold limits for linkage group construction.

The commands “group”, “sequence”, and “map” were used to develop the linkage groups and the position of markers on each chromosome. The commands “try” and “compare” were used to locate the unlinked markers on the chromosomes. The Kosambi (Kosambi, 1994) mapping function was used to convert the recombination fraction into cM values which were used as map distances. The linkage map was drawn by using Mapchart ver 2.1 (Voorrips, 2002).

The QTLNetwork 2 (http://ibi.zju.edu.cn/ software/) was employed to determine QTL for additive effects at individual locus, epistatic interactions between two different loci, and interaction between QTL and the environment (QTL x E) (Yang et al., 2005). The analyses were based on a mixed linear model (MLM) with 1 cM walking speed, 2D genome scan, which refer to map epistatic QTL with or without single locus effects with 1000 permutations to generate a threshold for the presence of QTL, QTL x E interactions and a genome-wide type I error rate of 5%, 1% and 0.1%. A QTL was considered to have been detected if the phenotype was associated with a marker locus at P < 0.005.

Data for QTL analysis were generated from each individual environment (three environments, see Experimental design) and the average of three environments (PD).

QTLs were designated according to the recommended international nomenclature for QTL in wheat (McIntosh et al., 1994).
**Statistical analysis**

ANOVA of phenotypic data was performed using SPSS13.0 software (SPSS, Chicago, USA). The distribution test was determined by using the Kolmogorov-Smirnov test. For the combined analysis across environments, the following linear model was used: $y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + (aD)_{ijk} + e_{ijk}$

Where, $a_i$, $b_j$ and $P_{ijk}$ represent the effects of genotype, environment and replication, respectively, $(ab)_ij$ indicates the effect of the genotype x environment interaction, $(aD)_{ijk}$, is the effect of the genotype x replication interaction across the three environments, and $e_{ijk}$ is the error. Basic sense heritability on across-year genotype mean and standard errors were calculated following Holland et al. (2003).

**Conclusion**

In this study, ten additive QTLs and 23 epistatic QTLs associated with FN were identified. To our knowledge, this is the first report on the detection of the four additive QTLs located nearby the $Wx$ genes, and the use of such unique RIL population with no PHS proved indirectly that the $Wx$ genes affect flour FN. We believe that it is possible to accelerate the development of elite cultivars with high flour FN and simultaneously low or no PHS using the molecular markers that are closely linked with the $Wx$ loci.

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