

## RESEARCH ARTICLE

# Genetic dissection reveals effects of interaction between high-molecular-weight glutenin subunits and waxy alleles on dough-mixing properties in common wheat

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### Abstract

The glutenin and waxy loci of wheat are important determinants of dough quality. This study was conducted to evaluate the effects of high-molecular-weight glutenin (HMW-GS) and waxy alleles on dough-mixing properties. Molecular mapping was used to investigate these effects on Mixograph properties in a population of 290 (Nuomai1 × Gaocheng8901) recombinant inbred lines (RILs) from three environments in the harvest years 2008, 2009 and 2011. The results indicated the following: (i) the *Glu-A1* and *Glu-D1* loci have greater impacts on Mixograph properties compared to the *Wx-1* loci and the effects of *Glu-D1d* and *Glu-D1h* on dough mixing are better than those of *Glu-D1f* and *Glu-D1new1* in this population; (ii) the interactions between the *Glu-1* and *Wx-1* loci affected some traits, especially the midline peak value (MPV), and the lack of *Wx-B1* or *Wx-D1* led to increased MPV for all types of *Glu-1* loci; and (iii) 30 quantitative-trait loci (QTL) over nine wheat chromosomes were identified with ICIM analysis based on the genetic map of 498 loci. Eight major QTL and 16 QTL in the *Glu-1* loci from the three environments were found. The major QTL clusters were associated with the *Glu-1* loci, and also were found in two regions on chromosome 3B and one region on chromosome 6A, which is one of the novel chromosome regions influencing dough-mixing strength. The two QTL for MPV are located around *Wx-B1* on chromosome 4A. *QMPT-1D.1*, *QMPI-1D.1* and *Q8MW-1D.1* were stable in different environments and could potentially be used in molecular marker-assisted breeding.

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### Introduction

Wheat quality is mainly influenced by protein and starch, which are responsible for end-use quality, and determine wheat flour and dough properties. The key components of the endosperm are gluten proteins, which are composed of glutenins and gliadins (Payne *et al.* 1987; Gupta *et al.* 1992). The starch granules are trapped in a protein matrix. Glutenin subunit composition and waxy subunit alleles, which are related to the amylose content (Yamamori and Quynh 2000; Yamamori 2009), are of particular interest to many wheat researchers and breeders.

Glutenin proteins are major factors affecting wheat flour dough viscoelastic properties and thus determine dough-mixing and bread-baking qualities. Glutenins are divided into two groups: high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs) (Payne and Corfield 1979). HMW-GSs are minor determinants in terms of quantity, but they substantially contribute to dough elasticity and baking quality. Thus these proteins, and especially their influence on dough quality, have been studied in detail by many researchers (Payne and Lawrence 1983; Hu *et al.* 2007; Zhang *et al.* 2009a). Correlations have been established between *Glu-1* alleles and bread-making quality. Notably, variation at the *Glu-D1* locus strongly affects the bread-making quality.

Starch contains two components: amylose and amylopectin. The formation of amylose is mainly catalysed by

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the granule-bound starch synthase (GBSS), which is encoded by the waxy genes (De Fekete *et al.* 1960) named *Wx-A1*, *Wx-B1* and *Wx-D1* (Murai *et al.* 1999). These genes are located on the short arm of chromosomes 7A (*Wx-A1* locus) and 7D (*Wx-D1* locus) and on the long arm of chromosome 4A (*Wx-B1* locus). The flour made from wheat cultivars with mutant waxy alleles lacking one or more waxy proteins exhibits unique starch qualities. The presence of three null alleles largely reduces the amylose content, leading to a greater peak paste viscosity, more breakdown and a lower final viscosity, all of which affect the white salted noodle-making quality (Nakamura *et al.* 1993).

Although variations in HMW-GS composition and waxy alleles have been investigated by many researchers, different cultivars or germplasms from different countries have been used (Graybosch *et al.* 1998; Branlard *et al.* 2003; Shan *et al.* 2007; Caballero *et al.* 2008). An overall evaluation of how variations in HMW-GSs and waxy proteins affect dough rheological properties in the same special recombinant inbred line (RIL) population derived from two Chinese parents, strong wheat Gaocheng8901 (strong gluten with good bread-making quality) and Nuomai 1 (waxy wheat with three null waxy alleles), has not been conducted.

Rheological characteristics are traditionally determined with a Mixograph (Zheng *et al.* 2009). Indeed, US breeding programmes typically prefer the Mixograph over other methods for assessing the functional properties of flour because it is quick (generally no more than 10 minutes), requires a relatively small amount of flour (10 g), and provides good data.

Dough rheological traits are generally controlled by several genes and cannot be fully explained by loci coding for storage proteins. They are also affected by environmental factors (Zheng *et al.* 2009, 2010). Better understanding of the genetic contributions to rheological traits is important for enhancing the development of superior or special wheat cultivars with different dough qualities.

With development of molecular-marker technologies and quantitative-trait analysis, the effects of quantitative-trait loci (QTL) associated with quality traits can be identified and estimated. For example, QTL for dough quality traits such as mixing time, mixing tolerance, dough tenacity and dough extensibility have been detected in wheat (Huang *et al.* 2006; Wu *et al.* 2008; Zhang *et al.* 2009b; Kerfal *et al.* 2010; Tsilo *et al.* 2011). Although QTL for the Mixograph properties have been studied and mapped on different chromosomes, such as 1A, 2A, 3A, 1B, 2B, 3B, 1D, 4D, 5B, 5D, 6B, 6D, 7A and 7D, there are no reports on QTL mapping for dough-mixing characteristics using this special RIL population.

Therefore, the objectives of this study were (i) to assess the effects of allelic variation at the *Glu-1* and *Wx-1* loci on the dough-mixing properties in the special RIL population; and (ii) to evaluate the additive effects on dough-mixing properties by QTL mapping using the same RIL population from three different environments.

## Materials and methods

### Plant materials

An RIL population of 290 lines was developed from a cross between the two winter wheat cultivars Nuomai 1 (female) and Gaocheng8901 (male). Briefly, the RIL population was developed by a single seed descent to the F<sub>10</sub> generation. Nuomai 1 (Jiangsu Baihuomai/Guandong107) carrying HMW-GSs or alleles of Ax-null, Bx7 + By8, and Dx2.2 + Dy12 at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively, was bred by China Agricultural University and released in 2005 in Beijing. It has 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. Gaocheng 8901 (77546-2/Lingzhang) has normal waxy alleles, was bred by Gaocheng Agricultural Science Research Institute and was released in 1998 in Hebei province. This cultivar carries HMW-GSs or alleles of Ax1, Bx7 + By8, and Dx5 + Dy10 at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. It exhibits high gluten strength and good bread-making quality.

### Field trials

The field trials were conducted over three years using a randomized complete block design. Two replicates were conducted at the experimental fields of Shandong Agricultural University, Tai'an City (36°57'N, 116°36'E), in the harvest years 2008 and 2009 and of Suzhou (33°38'N, 116°58'E), Anhui Province, in the harvest year 2011. For each environment, the RIL and parental lines were grown in 2-m-long four-row plots spaced 26 cm apart. During the growing season, field management was in accordance with the local practices, and the plants were not damaged by disease or insects.

### Mill flour

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

### Protein extraction and electrophoresis

The extraction and electrophoresis of HMW glutenin subunits by SDS-PAGE were conducted according to Deng *et al.* (2005). Chinese Spring and Marquis were used as controls. HMW-GSs were classified using the nomenclature of Payne and Lawrence (1983).

Starch granules were isolated from one seed of each wheat line according to the procedure reported by Zhao and Sharp (1996) with the following modifications. The seed was crushed and soaked overnight in 1 mL of water at 4°C. After shaking for 5 min, the samples were centrifuged for 3 min at

10,000 g. The pellet was added to 1 mL of washing buffer containing 0.138 M Tris-HCl, pH 6.8, 5.75% (w/v) SDS, 12.5% (v/v) 2-mercaptoethanol, and 25% (v/v) glycerol and then centrifuged for 3 min at 10,000 g. The supernatant was discarded. This step was repeated thrice. The starch was then washed twice with water, twice with acetone, and air dried. The Wx protein was extracted by adding protein solution buffer (100  $\mu$ L for approximately 5 mg of granules) containing 0.062 M Tris-HCl, pH 6.8, 2.3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.005% (w/v) bromophenol blue. The sample was heated in a boiling water bath for 15 min and then cooled for 5 min in ice water. The gel starch suspension was centrifuged at 10,000 g for 10 min.

A 30- $\mu$ L sample of the supernatant was used to separate the waxy proteins with SDS-PAGE (4.5% stacking gel and 15% separation gel). The gel was programmed to run at under 20 mA in the stacking gel. After the samples had run into the separation gel, 22 mA per gel was applied for approximately 17 h. The protein bands were detected by silver staining.

**Mixograph testing**

The dough-mixing properties were analysed for each sample with a 10 g Mixograph at room temperature according to the approved AACC method 54-40A (AACC Chemists 2004). Mixograph characteristics were determined using the software program Mixsmart v. 3.80 (AEW Consulting, Lincoln, USA) commercially available through the National Manufacturing Division of TCMCO (Lincoln, USA). An explanation of the Mixograph output was presented by Zheng *et al.* (2009). Briefly, all of the mixing characteristics were measured from the centre line (Mixograph midline) of the

mixograph. All the characteristics were automatically estimated by Mixsmart, including the midline peak time (MPT; min), midline peak value (MPV; %), midline peak width (MPW; %), midline peak integral (MPI; % torque  $\times$  min), and band width at 8 min (8MW). The MPI is determined from the area under the midline from the start to peak time (or the mean work input required to reach peak development time as a function of the mixing time and applied torque).

**Statistical analysis**

Analysis of variance (ANOVA) was carried out using the SAS program (SAS Institute, North Carolina, USA). The mean values were also analysed with SAS using Fisher’s least significant difference (LSD) procedure and Pearson correlations. Differences were considered significant at  $P < 0.05$ .

The molecular genetic map was constructed by MAP-MAKER/EXP v. 3.0b (Lincoln *et al.* 1992). A recombination frequency of 0.4 and an LOD value 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 mapping function (Kosambi 1994) was used to convert the recombination fraction into cM values as map distances. The linkage map was drawn by Mapchart v. 2.1 (Voorrips 2002).

The QTL analysis was performed and the main effect QTL (M-QTL) were identified with inclusive composite interval mapping (QTL IciMapping v. 3.1) (Wang *et al.* 2007; Li *et al.* 2008; Wang 2009; Zhang *et al.* 2010) from the three

**Table 1.** Means, standard deviations and ranges for observed dough mixing characteristics for the RIL population ( $n = 267$ ) from three environments.

| E | Trait | Mean   | Variance | Std. error | Skewness | Kurtosis | Range        | Nuomai1 | Gaocheng8901 |
|---|-------|--------|----------|------------|----------|----------|--------------|---------|--------------|
| 1 | MPT   | 2.91   | 0.64     | 0.80       | 0.43     | -0.08    | 1.5-5.53     | 1.82    | 4.87         |
|   | MPV   | 61.90  | 25.79    | 5.08       | 0.15     | 0.07     | 48.5-77.92   | 67.24   | 58.64        |
|   | MPW   | 23.55  | 11.23    | 3.35       | -0.10    | 0.37     | 11.43-32.81  | 22.74   | 23.92        |
|   | MPI   | 146.32 | 1645.68  | 40.57      | 0.52     | 0.23     | 68.14-300.94 | 102.91  | 238.30       |
|   | 8MW   | 6.05   | 9.05     | 3.01       | 2.31     | 6.02     | 0.16-20.72   | 4.87    | 13.90        |
| 2 | MPT   | 3.90   | 1.87     | 1.37       | 0.77     | 0.57     | 1.5-9.01     | 3       | 4.51         |
|   | MPV   | 61.06  | 27.89    | 5.28       | -0.02    | -0.61    | 47.26-73.89  | 62.48   | 62.63        |
|   | MPW   | 23.61  | 29.91    | 5.47       | 1.18     | 2.59     | 12.95-49.48  | 22.91   | 25.88        |
|   | MPI   | 192.7  | 4125.81  | 64.23      | 0.72     | 0.52     | 74.99-426.63 | 160.67  | 221.46       |
|   | 8MW   | 9.21   | 25.20    | 5.02       | 2.97     | 13.12    | 0.43-43.98   | 7.65    | 13.55        |
| 3 | MPT   | 2.66   | 0.48     | 0.70       | 0.71     | 0.49     | 1.5-5.15     | 2.04    | 3.92         |
|   | MPV   | 63.58  | 35.89    | 5.99       | 0.61     | 0.90     | 46.98-86.97  | 69.72   | 61.64        |
|   | MPW   | 25.81  | 23.68    | 4.87       | 0.87     | 2.03     | 11.22-44.35  | 29.73   | 23.53        |
|   | MPI   | 135.38 | 1504.49  | 38.79      | 0.77     | 0.87     | 56.30-283.84 | 120.02  | 192.78       |
|   | 8MW   | 9.88   | 22.80    | 4.77       | 1.57     | 2.92     | 4.21-33.64   | 7.37    | 16.61        |

E, environment; 1, Tai’an location in 2008; 2, Tai’an location in 2009; 3, Suzhou location in 2011.

MPT, Mixograph peak time; MPW, Mixograph band width at MPT; MPV, Mixograph midline peak value; MPI, Mixograph midline peak integral; 8MW, Mixograph band width at 8 minutes.

different environments. The walking speed for all of the QTL was 1.0 cM. A LOD score of 2.5 was used for declaring the presence of a putative QTL. The threshold LOD scores for detection of a QTL were calculated based on 1000 permutations.

QTL were designated according to the QTL + trait + chromosome format in accordance with the recommended international nomenclature for QTL in wheat (McCouch et al. 1997). If more than one QTL was found on a chromosome, then a serial number was added after the chromosome number (e.g. *QZsv-1B.1*, *QZsv-1B.2*).

## Results

### Trait means and their correlations

All the evaluated traits exhibited approximately continuous variation in each of the environments (table 1; see table 1 in electronic supplementary material at <http://www.ias.ac.in/jgenet/>). Transgressive segregation was observed on both the high and low sides for all traits in this population, indicating that alleles with positive effects were contributed from both parents. According to the outputs of the ANOVA, the environment main effects were highly significant ( $P < 0.001$ ) for all the traits, but the genotype main effects were highly significant for MPT, MPV and MPI, but not for MPW and 8MW (table 2).

The correlations among dough-mixing characteristics are summarized in table 3. Strong correlations were observed among the mixing time parameters (MPT, MPV, MPW, MPI and 8MW). MPT was significantly negatively correlated with MPV and MPW but was positively correlated with MPI and 8MW. Significant positive correlation was observed between MPV and MPW. These results indicate that these parameters share some genes in common.

### Effects of HMW-GS and *Wx* allelic diversity on mixing properties

High level of diversity was observed for *Glu-A1* and *Glu-D1* and for the three *Wx* loci in this population (table 4). There

**Table 3.** Correlation analysis of mixing characteristics.

|     | MPV     | MPW     | MPI    | 8MW    |
|-----|---------|---------|--------|--------|
| MPT | -0.183* | -0.167* | 0.96*  | 0.572* |
| MPV | 1       | 0.588*  | 0.012  | 0.054  |
| MPW |         | 1       | -0.065 | 0.212* |
| MPI |         |         | 1      | 0.589* |

MPT: midline peak time; MPV: midline peak value; MPW: midline peak width; MPI: midline peak intergral; 8MW: band width at 8 minutes

were two (a and c) and four alleles (d, f, h and new1:2.2 + 10) at *Glu-A1* and *Glu-D1*, respectively. For the *Glu-A1* loci, significant differences were observed for MPT, MPV, MPI and 8MW except MPW between *Glu-A1a* and *Glu-A1c*. The contribution of subunit 1 to the mixing properties is greater than that of a null allele at *Glu-A1* loci. However, at *Glu-D1* loci, MPT was significantly affected by *Glu-D1* allelic diversity. Significant differences were found between *Glu-D1d* and other three alleles. *Glu-D1h* had higher MPT than *Glu-D1f* and *Glu-D1new1*, but the difference was not significant. The effects of *Glu-D1* loci on MPI and 8MW were the same as those on MPT in this population. For MPV, *Glu-D1f* was significantly different from *Glu-D1d* and *Glu-D1h*, but no significant differences were observed from *Glu-D1new1*. MPW was almost not affected by *Glu-D1* loci, as no differences were observed among the four alleles.

There were no significant differences in the mixing properties between *Wx-A1a* and *Wx-A1b* at the *Wx-A1* loci. Only MPV was significantly affected by *Wx-B1* loci, and *Wx-B1b* had a greater effect than *Wx-B1a*. These results indicate that deletion of *Wx-B1* significantly contributes to MPV. Significant differences were observed in MPT and MPI at *Wx-D1* loci. *Wx-D1a* exhibited greater effects than *Wx-D1b*.

So the effects of HMW-GS allelic diversity on the mixing characteristics were stronger than those of *Wx* loci.

**Table 2.** Effects of environment and genotype on Mixograph properties.

| Trait | Factor | d.f. | Type III SS | Mean square | F value | P       |
|-------|--------|------|-------------|-------------|---------|---------|
| MPT   | E      | 2    | 202.22      | 101.1       | 118.83  | <0.0001 |
|       | G      | 266  | 323.2       | 1.2         | 1.43    | <0.0001 |
| MPV   | E      | 2    | 727.2       | 363.6       | 15.58   | <0.0001 |
|       | G      | 266  | 10805.85    | 40.6        | 1.74    | <0.0001 |
| MPW   | E      | 2    | 792.1       | 396         | 19.97   | <0.0001 |
|       | G      | 266  | 6300.4      | 23.69       | 1.19    | 0.048   |
| MPI   | E      | 2    | 439924.9    | 219962.5    | 109.92  | <0.0001 |
|       | G      | 266  | 817459      | 3073.15     | 1.54    | <0.0001 |
| 8MW   | E      | 2    | 2046.35     | 1023.2      | 59.72   | <0.0001 |
|       | G      | 266  | 5820.1      | 21.9        | 1.28    | 0.01    |

E: environment; G: genotype; MPT: midline peak time; MPV: midline peak value; MPW: midline peak width; MPI: midline peak integral; 8MW: band width at 8 minutes

**Table 4.** Effects of glutenin loci and waxy loci on Mixograph properties in the RIL population.

| Locus         | Allele <sup>a</sup> | n   | Mixograph properties <sup>b</sup> |         |        |         |       |
|---------------|---------------------|-----|-----------------------------------|---------|--------|---------|-------|
|               |                     |     | MPT                               | MPV     | MPW    | MPI     | 8MW   |
| <i>Glu-A1</i> | <i>a</i>            | 142 | 3.11a                             | 62.74a  | 23.93a | 158.10a | 6.49a |
|               | <i>c</i>            | 125 | 2.75b                             | 60.69b  | 23.14a | 135.54b | 5.68b |
| <i>Glu-D1</i> | <i>d</i>            | 162 | 3.28a                             | 61.15b  | 23.10a | 163.47a | 6.72a |
|               | <i>f</i>            | 91  | 2.41b                             | 63.06a  | 24.37a | 122.66b | 5.13b |
|               | <i>h</i>            | 8   | 2.67b                             | 58.619b | 22.69a | 128.14b | 5.36b |
|               | <i>new1</i>         | 6   | 2.33b                             | 63.49a  | 24.29a | 121.74b | 5.46b |
| <i>Wx-A1</i>  | <i>a</i>            | 139 | 2.97a                             | 61.23a  | 23.68a | 147.82a | 6.28a |
|               | <i>b</i>            | 128 | 2.91a                             | 62.38a  | 24.98a | 147.33a | 5.93a |
| <i>Wx-B1</i>  | <i>a</i>            | 146 | 3.00a                             | 60.65b  | 23.26a | 147.71a | 6.09a |
|               | <i>b</i>            | 121 | 2.87a                             | 63.14a  | 23.89a | 147.39a | 6.12a |
| <i>Wx-D1</i>  | <i>a</i>            | 132 | 3.05a                             | 61.25a  | 23.44a | 151.07a | 6.16a |
|               | <i>b</i>            | 135 | 2.84b                             | 62.29a  | 23.65a | 144.14b | 6.03a |

<sup>a</sup>Subunit allele designations for the HMW-GS loci are *Glu-A1*: *a*, 1; *c*, null; *Glu-D1*: *d*, 5 + 10; *f*, 2.2 + 12; *h*, 5 + 12; *new1*, 2.2 + 10. For *Wx-A1*, *Wx-B1* and *Wx-D1* loci: *a*, wild type; *b*, mutation (no protein).

<sup>b</sup>Different letters after the means indicate significant difference at  $P < 0.05$ .

**Effects of HMW-GS and Wx subunit combinations on the mixing characteristics**

There were significant differences between the combination (1/7 + 8/5 + 10) and the other three combinations for MPT and MPI (table 5). The combination (1/7 + 8/5 + 10) had the highest value for MPT and MPI, while the combination (null/7 + 8/2.2 + 12) showed the lowest value. When *Glu-A1* and *Glu-B1* had the same combinations, *Glu-D1d* showed better effect on MPT and MPI than *Glu-D1f*.

For MPV and MPW, the combination (1/7 + 8/2.2 + 12) with the highest value showed significant differences from (1/7 + 8/5 + 10), (null/7 + 8/5 + 10) and (null/7 + 8/2.2 + 12). No significant differences were observed between the combination (null/7 + 8/5 + 10) and (null/7 + 8/2.2 + 12) for MPW.

For 8MW, significant difference was seen between the combination (1/7 + 8/5 + 10) with the highest value and (null/7 + 8/2.2 + 12) with the lowest value, but no significant difference with the combination (null/7 + 8/5 + 10).

**Table 5.** Effects of HMW-GS and Wx subunit combinations on Mixograph properties in the RIL population.

| <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> | n  | Mixograph properties |         |         |          |        |
|---------------|---------------|---------------|----|----------------------|---------|---------|----------|--------|
|               |               |               |    | MPT                  | MPV     | MPW     | MPI      | 8MW    |
| 1             | 7 + 8         | 5 + 10        | 88 | 3.44a                | 62.25b  | 23.52b  | 173.78a  | 7.17a  |
| 1             | 7 + 8         | 2.2 + 12      | 46 | 2.56c                | 64.29a  | 25.05a  | 132.62c  | 5.32bc |
| Null          | 7 + 8         | 5 + 10        | 74 | 3.09b                | 59.84c  | 22.59b  | 151.22b  | 6.18ab |
| Null          | 7 + 8         | 2.2 + 12      | 45 | 2.26d                | 61.81b  | 23.68b  | 112.48d  | 4.94c  |
| 1             | 7 + 8         | 5 + 12        | 6  | 2.83                 | 58.02   | 21.41   | 134.64   | 5.8    |
| 1             | 7 + 8         | 2.2 + 10      | 2  | 2.45                 | 62.87   | 23.26   | 124.58   | 5.47   |
| Null          | 7 + 8         | 5 + 12        | 2  | 2.2                  | 60.41   | 26.55   | 108.65   | 4.01   |
| Null          | 7 + 8         | 2.2 + 10      | 4  | 2.27                 | 63.8    | 24.81   | 120.32   | 5.46   |
| <i>Wx-A1</i>  | <i>Wx-B1</i>  | <i>Wx-D1</i>  |    |                      |         |         |          |        |
| <i>a</i>      | <i>a</i>      | <i>a</i>      | 42 | 3.12ab               | 60.13c  | 23.59ab | 152.08ab | 6.53ab |
| <i>a</i>      | <i>a</i>      | <i>b</i>      | 38 | 2.84b                | 60.28c  | 23.40ab | 138.85b  | 5.86ab |
| <i>a</i>      | <i>b</i>      | <i>a</i>      | 30 | 2.94b                | 62.1bc  | 23.33ab | 148.03ab | 6.32ab |
| <i>b</i>      | <i>a</i>      | <i>a</i>      | 25 | 3.35a                | 60.46c  | 22.60b  | 165.28a  | 6.85a  |
| <i>a</i>      | <i>b</i>      | <i>b</i>      | 29 | 2.95b                | 63.16ab | 24.53a  | 150.86ab | 6.41ab |
| <i>b</i>      | <i>a</i>      | <i>b</i>      | 41 | 2.82b                | 61.64bc | 23.20ab | 140.754b | 5.4b   |
| <i>b</i>      | <i>b</i>      | <i>a</i>      | 35 | 2.84b                | 62.44bc | 23.97ab | 142.32b  | 5.32b  |
| <i>b</i>      | <i>b</i>      | <i>b</i>      | 27 | 2.74b                | 65.18a  | 23.72ab | 149.52ab | 6.65ab |

Different letters after the means indicate significant difference at  $P < 0.05$ .

There was no significant difference between (1/7 + 8/2.2 + 12) and (null/7 + 8/2.2 + 12).

Although four new combinations were found in this population, the number of samples per combination was so small that we could not carry out the analysis of difference using these data (table 5).

Of the eight combinations, (b/a/a) showed the highest value for MPT, MPI and 8MW. Significant differences were observed for MPT between combination (b/a/a) and six other combinations, but not with (a/a/a). The combination (b/a/a) showed significant differences from (b/a/b) and (b/b/a) for MPI and 8MW. For MPV, the combination (b/b/b) was significantly different from (a/a/a), (a/a/b), (a/b/a), (b/a/a), (b/a/b) and (b/b/a). Of the eight combinations, (a/b/b)

exhibited the highest value for MPW and was significantly different from (b/a/a).

#### Effect of interactions between *Glu-1* and *Wx* loci on mixing properties

Interactions between *Glu-1* and *Wx* loci had no significant influence on almost all the Mixograph properties except MPV (table 6). Interaction between *Glu-1* and *Wx-B1* loci significantly affected MPV. Combination *Glu-1a/Wx-B1b* was always associated with higher values of MPV than *Glu-1a/Wx-B1a*, so did *Glu-1c/Wx-B1* loci. Interaction between *Glu-1c* and *Wx-D1* loci significantly influenced MPV. Combination *Glu-1c/Wx-D1b* led to a higher value of MPV than *Glu-1c/Wx-D1a*. Combination *Glu-1a/Wx-1* (*Wx-A1*, *Wx-B1* and *Wx-D1*) was always associated with higher values of Mixograph traits than combination *Glu-1c/Wx-1*.

No significant interactions were observed between *Glu-D1* (*Glu-D1d*, *Glu-D1f*, *Glu-D1h*, *Glu-D1new1*) and *Wx-A1* (*Wx-A1a*, *Wx-A1b*) alleles for most of the Mixograph traits (table 6). However, important interactions were found between *Glu-D1f* and *Wx-A1* for MPV and 8MW. Significant differences for MPV and 8MW were observed between combinations *Glu-D1f/Wx-A1b* and *Glu-D1f/Wx-A1a*.

There were no significant interactions between *Glu-D1h* and *Wx-B1* for the Mixograph traits (table 6). However, significant interactions were observed between *Glu-D1f* and *Wx-B1* for MPV, MPW and 8MW, and combination *Glu-D1f/Wx-B1b* showed a higher value of MPV, MPW and

**Table 6.** Mean values of Mixograph properties for combinations of alleles of *Glu-1* loci and *Wx* loci in the RIL population.

| Locus 1           | Locus 2       | Mixograph characteristics |        |        |         |       |
|-------------------|---------------|---------------------------|--------|--------|---------|-------|
|                   |               | MPT                       | MPV    | MPW    | MPI     | 8MW   |
| <i>Glu-1a</i>     | <i>Wx-1a</i>  | 3.16a                     | 62.08a | 23.78a | 157.84a | 6.62a |
|                   | <i>Wx-1b</i>  | 3.06a                     | 63.46a | 24.09a | 158.38a | 6.35a |
| <i>Glu-1c</i>     | <i>Wx-1a</i>  | 2.75a                     | 60.26a | 23.56a | 135.37a | 5.89a |
|                   | <i>Wx-1b</i>  | 2.74a                     | 61.15a | 22.63a | 135.86a | 5.45a |
| <i>Glu-1a</i>     | <i>Wx-B1a</i> | 3.18a                     | 61.68b | 23.78a | 158.20a | 6.59a |
|                   | <i>Wx-B1b</i> | 3.03a                     | 64.07a | 24.11a | 157.97a | 6.36a |
| <i>Glu-1c</i>     | <i>Wx-B1a</i> | 2.80a                     | 59.44b | 22.65a | 135.35a | 5.51a |
|                   | <i>Wx-B1b</i> | 2.69a                     | 62.13a | 23.65a | 135.89a | 5.87a |
| <i>Glu-1a</i>     | <i>Wx-D1a</i> | 3.23a                     | 62.46a | 23.66a | 162.61a | 6.65a |
|                   | <i>Wx-D1b</i> | 2.99a                     | 63.03a | 24.20a | 153.32a | 6.32a |
| <i>Glu-1c</i>     | <i>Wx-D1a</i> | 2.83a                     | 59.76b | 23.17a | 136.79a | 5.70a |
|                   | <i>Wx-D1b</i> | 2.68a                     | 61.52a | 23.07a | 134.54a | 5.65a |
| <i>Glu-D1d</i>    | <i>Wx-A1a</i> | 3.32a                     | 61.03a | 23.56a | 164.50a | 7.17a |
|                   | <i>Wx-A1b</i> | 3.26a                     | 60.49a | 22.28b | 161.91a | 6.23a |
| <i>Glu-D1f</i>    | <i>Wx-A1a</i> | 2.47a                     | 61.55b | 24.30a | 122.39a | 4.85b |
|                   | <i>Wx-A1b</i> | 2.40a                     | 65.04a | 24.86a | 126.52a | 5.59a |
| <i>Glu-D1h</i>    | <i>Wx-A1a</i> | 3.24a                     | 60.68a | 23.28a | 160.20a | 7.03a |
|                   | <i>Wx-A1b</i> | 3.05a                     | 62.88a | 23.66a | 156.86a | 5.96a |
| <i>Glu-D1new1</i> | <i>Wx-A1a</i> | 2.14a                     | 63.01a | 22.70a | 108.70a | 4.66a |
|                   | <i>Wx-A1b</i> | 2.36a                     | 62.96a | 24.27a | 121.10a | 5.30a |
| <i>Glu-D1d</i>    | <i>Wx-B1a</i> | 3.44a                     | 59.85b | 23.00a | 168.19a | 7.23a |
|                   | <i>Wx-B1b</i> | 3.07b                     | 62.06a | 22.78a | 156.02a | 5.93a |
| <i>Glu-D1f</i>    | <i>Wx-B1a</i> | 2.46a                     | 61.79b | 23.80b | 122.39a | 4.81b |
|                   | <i>Wx-B1b</i> | 2.41a                     | 64.93a | 25.57a | 126.76a | 5.69a |
| <i>Glu-D1h</i>    | <i>Wx-B1a</i> | 3.00a                     | 60.95a | 22.98a | 148.17a | 5.62a |
|                   | <i>Wx-B1b</i> | 3.28a                     | 62.16a | 23.80a | 166.87a | 7.29a |
| <i>Glu-D1new1</i> | <i>Wx-B1a</i> | 2.36a                     | 60.35b | 23.26a | 116.19a | 5.05a |
|                   | <i>Wx-B1b</i> | 2.18a                     | 65.35a | 23.93a | 115.60a | 5.01a |
| <i>Glu-D1d</i>    | <i>Wx-D1a</i> | 3.46a                     | 60.36a | 22.45a | 170.50a | 7.08a |
|                   | <i>Wx-D1b</i> | 3.10b                     | 61.20a | 23.42a | 155.24b | 6.27a |
| <i>Glu-D1f</i>    | <i>Wx-D1a</i> | 2.40a                     | 62.47a | 25.29a | 119.69a | 4.78a |
|                   | <i>Wx-D1b</i> | 2.47a                     | 63.57a | 24.04a | 127.41a | 5.46a |
| <i>Glu-D1h</i>    | <i>Wx-D1a</i> | 3.17a                     | 60.59a | 23.29a | 156.61a | 6.46a |
|                   | <i>Wx-D1b</i> | 3.14a                     | 62.89a | 23.63a | 161.35a | 6.70a |
| <i>Glu-D1new1</i> | <i>Wx-D1a</i> | 2.26a                     | 64.76a | 24.05a | 116.91a | 4.92a |
|                   | <i>Wx-D1b</i> | 2.27a                     | 61.00a | 23.13a | 114.73a | 5.14a |

Different letters after the means indicate significant difference at  $P < 0.05$ .

**Table 7.** Effects of interactions between HMW-GS and *Wx* subunits on mixing properties in the RIL population using the generalized linear model (GLM) of SAS.

| Trait |                | HMW-GS   | <i>Wx</i> | HMW-GS × <i>Wx</i> |
|-------|----------------|----------|-----------|--------------------|
| MPT   | d.f.           | 7        | 7         | 44                 |
|       | Type III SS    | 52.97    | 4.58      | 20.74              |
|       | Mean square    | 7.57     | 0.65      | 0.47               |
|       | <i>F</i> value | 16.89    | 1.46      | 1.05               |
| MPV   | <i>P</i>       | <0.0001  | 0.18      | 0.39               |
|       | Type III SS    | 661.86   | 644.43    | 1087.32            |
|       | Mean square    | 94.55    | 92.06     | 24.71              |
|       | <i>F</i> value | 4.61     | 4.49      | 1.21               |
| MPW   | <i>P</i>       | <0.0001  | <0.0001   | 0.19               |
|       | Type III SS    | 213.51   | 58.26     | 475.92             |
|       | Mean square    | 30.50    | 8.32      | 10.82              |
|       | <i>F</i> value | 2.89     | 0.79      | 1.02               |
| MPI   | <i>P</i>       | 0.01     | 0.60      | 0.44               |
|       | Type III SS    | 83752.95 | 4891.83   | 61492.40           |
|       | Mean square    | 11964.71 | 698.83    | 1397.55            |
|       | <i>F</i> value | 10.44    | 0.61      | 1.22               |
| 8MW   | <i>P</i>       | <0.0001  | 0.75      | 0.18               |
|       | Type III SS    | 90.00    | 37.77     | 459.42             |
|       | Mean square    | 12.86    | 5.40      | 10.44              |
|       | <i>F</i> value | 1.47     | 0.62      | 1.2                |
|       | <i>P</i>       | 0.18     | 0.74      | 0.20               |

8MW than *Glu-D1f/Wx-B1a*. Only the interactions between *Glu-D1new1* and *Wx-B1* significantly affected MPV. Combination *Glu-D1d/Wx-B1a* gave significantly higher MPT than *Glu-D1d/Wx-B1b*, but the results were opposite for MPV.

No significant interactions between *Glu-D1* and *Wx-D1* were observed for most of the Mixograph traits. Only the combination *Glu-D1d/Wx-D1a* gave significantly higher values for MPT and MPI than *Glu-D1d/Wx-D1b*.

Therefore, interactions between different HMW-GS and Wx protein subunit alleles affect some Mixograph traits, especially MPV. Deletions of *Wx-B1* and *Wx-D1* increase MPV in all types of HMW-GS.

Interactions between HMW-GS and Wx subunit combinations (table 7) indicated that HMW-GS combinations significantly affect MPT, MPV and MPI, but Wx combinations significantly influence only MPV. No significant effects were observed for HMW-GS × Wx on the Mixograph traits.

**QTL mapping**

Of the 1052 markers, 498 (47.3%) were found to be polymorphic in the parental lines; these markers were used for linkage analysis and mapping of the mixograph traits.

**Table 8.** Summary of QTL for dough-mixing properties using 256 recombinant inbred lines evaluated from three environments.

| Trait | QTL              | Flanking markers    | Position | Year | LOD  | PVE <sup>a</sup> (%) | Add <sup>b</sup> |
|-------|------------------|---------------------|----------|------|------|----------------------|------------------|
| MPT   | <i>QMPT-1A</i>   | wPt9757–Glu-A1      | 83       | 2008 | 4.0  | 4.44                 | -0.17            |
|       |                  |                     |          | 2011 | 4.8  | 5.72                 | -0.17            |
|       | <i>QMPT-1D.1</i> | Glu-D1–wPt3743      | 107      | 2008 | 10.3 | 12.55                | -0.28            |
|       |                  |                     |          | 2009 | 14.7 | 22.92                | -0.65            |
|       |                  |                     |          | 2011 | 22.6 | 30.95                | -0.39            |
|       | <i>QMPT-1D.2</i> | wPt8854–wPt0077     | 114      | 2008 | 2.5  | 2.84                 | -0.13            |
|       |                  |                     |          | 2008 | 41   | 2.8                  | 2.91             |
|       | <i>QMPT-3B</i>   | wPt9432–wPt9510     | 143      | 2008 | 3.7  | 3.95                 | -0.16            |
|       |                  |                     |          | 2009 | 131  | 4.5                  | 11.51            |
|       | <i>QMPT-6A</i>   | wPt664792–wPt730772 | 163      | 2009 | 5.3  | 7.62                 | 0.38             |
| 2011  |                  |                     |          | 158  | 3.0  | 3.40                 | -0.13            |
| MPV   | <i>QMPV-1A.1</i> | wPt664666–wPt9757   | 78       | 2008 | 4.6  | 7.06                 | -1.35            |
|       |                  |                     |          | 2011 | 4.3  | 6.53                 | -1.53            |
|       | <i>QMPV-1A.2</i> | wPt9757–Glu-A1      | 79       | 2009 | 5.2  | 7.74                 | -1.47            |
|       |                  |                     |          | 2008 | 88   | 4.2                  | 6.66             |
|       | <i>QMPV-4A.1</i> | wPt664948–Wx-B1     | 88       | 2009 | 3.1  | 5.06                 | 1.19             |
|       |                  |                     |          | 2011 | 101  | 3.3                  | 5.92             |
| MPW   | <i>QMPV-4A.2</i> | Wx-B1–wPt0105       | 101      | 2008 | 2.5  | 6.96                 | 2.83             |
|       |                  |                     |          | 2009 | 161  | 5.7                  | 35.66            |
|       | <i>QMPW-3B.1</i> | wPt5870–wPt3620     | 153      | 2008 | 6.0  | 7.06                 | -10.77           |
|       |                  |                     |          | 2009 | 83   | 4.3                  | 5.83             |
| MPI   | <i>QMPI-1A</i>   | wPt9757–Glu-A1      | 83       | 2011 | 3.1  | 3.78                 | -7.53            |
|       |                  |                     |          | 2008 | 106  | 26.0                 | 37.02            |
|       | <i>QMPI-1D.1</i> | Glu-D1–wPt3743      | 106      | 2009 | 15.0 | 22.93                | -30.78           |
|       |                  |                     |          | 2011 | 110  | 16.8                 | 22.46            |
|       | <i>QMPI-1D.2</i> | wPt3743–wPt666719   | 110      | 2008 | 3.1  | 3.40                 | -7.47            |
|       |                  |                     |          | 2008 | 143  | 2.9                  | 3.28             |
|       | <i>QMPI-3B</i>   | wPt9432–wPt9510     | 41       | 2009 | 3.0  | 4.03                 | -12.89           |
|       |                  |                     |          | 2009 | 162  | 4.8                  | 6.48             |
|       | <i>QMPI-6A</i>   | wPt664792–wPt730772 | 143      | 2009 | 4.8  | 6.48                 | 16.37            |
|       |                  |                     |          | 2011 | 50   | 6.2                  | 7.51             |
|       | <i>QMPI-1B</i>   | wPt6442–wPt3824     | 162      | 2011 | 6.2  | 7.51                 | -10.72           |
|       |                  |                     |          | 2011 | 64   | 3.3                  | 4.11             |
|       | <i>QMPI-4D.1</i> | wPt2379–wPt666601   | 50       | 2011 | 3.3  | 4.11                 | 7.86             |
|       |                  |                     |          | 2011 | 64   | 3.3                  | 4.11             |
|       | <i>QMPI-4D.2</i> | wPt666601–cfG-71    | 64       | 2011 | 3.3  | 4.11                 | 7.86             |
|       |                  |                     |          | 2011 | 64   | 3.3                  | 4.11             |
| 8MW   | <i>Q8MW-1D.1</i> | Glu-D1–wPt3743      | 108      | 2008 | 6.1  | 10.70                | -0.99            |
|       |                  |                     |          | 2009 | 113  | 5.1                  | 8.16             |
|       | <i>Q8MW-1D.2</i> | wPt3743–wPt666719   | 113      | 2011 | 58.2 | 160.86               | -6.05            |
|       |                  |                     |          | 2011 | 239  | 2.7                  | 4.39             |
|       | <i>Q8MW-1D.3</i> | wPt665204–wPt671415 | 239      | 2011 | 2.7  | 4.39                 | -0.10            |
|       |                  |                     |          | 2008 | 41   | 3.0                  | 4.65             |
|       | <i>Q8MW-3B.1</i> | wPt9432–wPt9510     | 41       | 2008 | 3.0  | 4.65                 | -0.65            |
|       |                  |                     |          | 2011 | 151  | 6.1                  | 35.66            |
|       | <i>Q8MW-3B.2</i> | wPt666008–wPt5870   | 151      | 2011 | 6.1  | 35.66                | -4.98            |
|       |                  |                     |          | 2008 | 143  | 5.2                  | 8.34             |
|       | <i>Q8MW-6A</i>   | wPt664792–wPt730772 | 143      | 2009 | 3.4  | 7.23                 | -1.35            |
|       |                  |                     |          | 2009 | 139  | 3.4                  | 7.23             |
|       | <i>Q8MW-1B</i>   | wPt6442–wPt3824     | 163      | 2009 | 5.3  | 8.40                 | 1.46             |
|       |                  |                     |          | 2009 | 240  | 3.7                  | 5.86             |
|       | <i>Q8MW-2A</i>   | wPt669693–wPt9951   | 240      | 2009 | 3.7  | 5.86                 | 2.01             |
|       |                  |                     |          | 2009 | 22   | 3.2                  | 4.89             |
|       | <i>Q8MW-6B</i>   | wPt3060–wPt664174   | 22       | 2009 | 3.2  | 4.89                 | 1.14             |

<sup>a</sup>Phenotypic variance explained for the QTL.

<sup>b</sup>Additive effect: positive values indicate increasing effect of Nuomail alleles, while negative values indicate increasing effect of Gaocheng8901 alleles.

A genetic linkage map was produced using 498 markers, including 479 DArT, 14 SSR, two HMW-GS and three Wx protein makers. These covered 4229.7 cM, with an average distance of 9.77 cM, and mapped to 21 chromosomes. There were large gaps in 1A, 6A and 7D, which formed a linkage

group. Therefore, 24 linkage groups were constructed. The three genomes A, B and D had 211, 166 and 121 markers, respectively.

A total of 30 QTL for Mixograph characteristics over nine wheat chromosomes (1A, 1B, 1D, 2A, 3B, 4A, 4D, 6A and

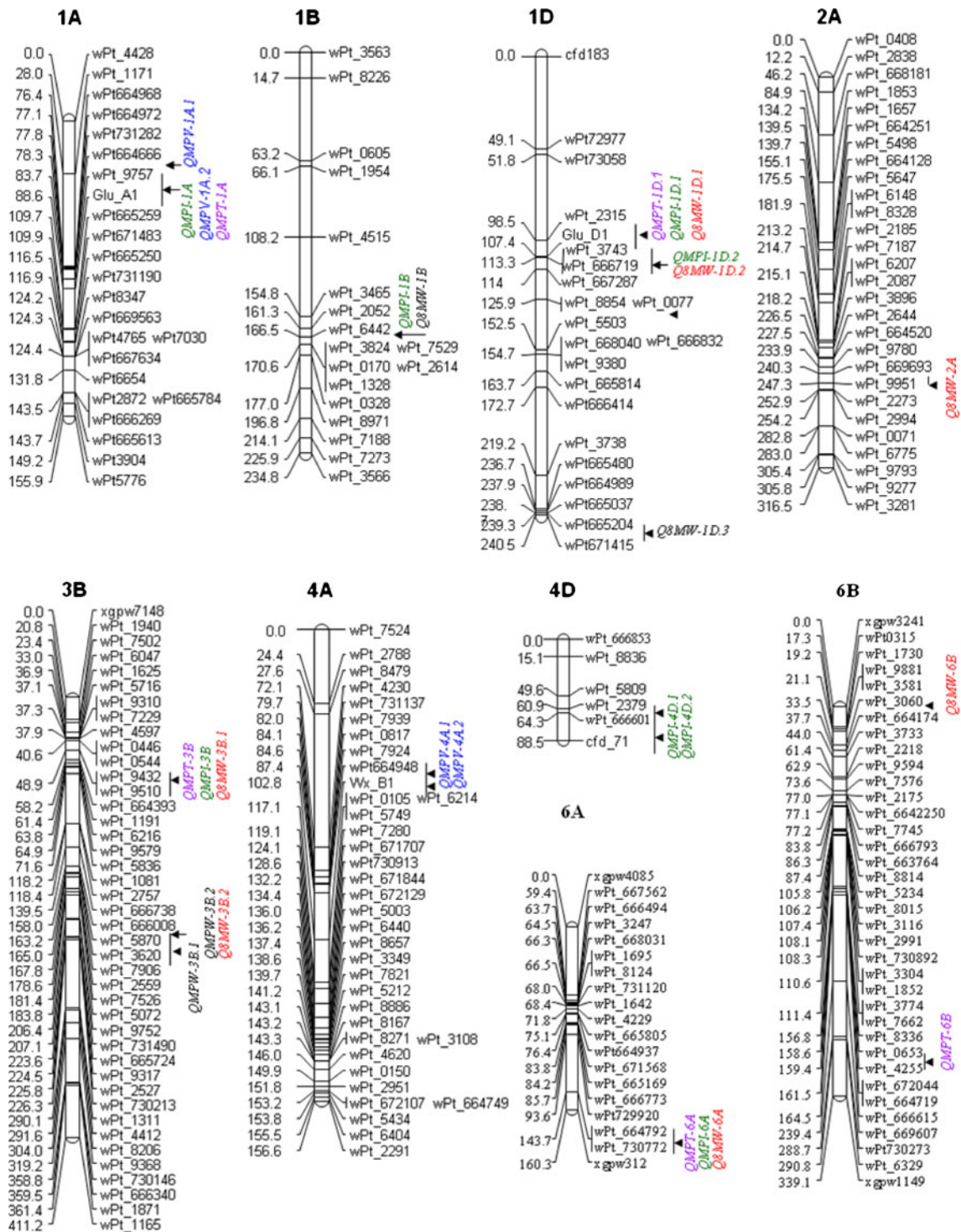


Figure 1. QTL detected for Mixograph characteristics and collocated QTL for five traits in the Nuomai/Gaocheng8901 RIL population.



6B) were detected by ICIM analysis (table 8). Of these, two (*QMPT-1D.1* and *QMPI-1A*) were consistent over three years, and eight were consistent over two years. Their map positions are shown in figure 1. Seven major QTL were identified on chromosomes 1D, 6A and 3B. Five major QTL clusters on chromosomes 1A, 1D, 3B and 6A were found, mainly influencing MPT, MPI, MPW and 8MW. MPV was mainly affected by QTL clusters on chromosomes 1A and 4A. A QTL cluster was identified in the wPt2379–cfG-71 interval on chromosome 4D that influenced MPI.

*QMPT-1D.1*, *QMPI-1D.1*, *QMPI-1D.2*, *Q8MW-1D.1* and *Q8MW-1D.2* from Gaocheng8901 (Suzhou field trial 2011) in association with *Glu-D1* had larger effects on MPT, MPI and 8MW than other QTL, and explain 35.66% of the phenotypic variance. These results indicate that *Glu-D1* loci are very important for MPT, MPI and 8MW. Two minor QTL (*QMPV-4A.1* and *QMPV-4A.2*) were important for MPV. Their association with *Wx-B1* from Nuomai1 caused significant effects on MPV.

## Discussion

### Effects of glutenin and *Wx* loci

Glutenin proteins significantly affect viscoelastic dough properties of wheat flour, thus influencing dough-mixing and bread-making qualities. The relationship between glutenin loci and dough quality has been extensively studied, and the allelic ranks for quality measurements are variable, except for *Glu-D1d* and *Glu-D1a*. These conclusions were obtained by statistical analysis using different wheat varieties with various HMW-GS compositions. Our results agreed with some of these previous studies, but differed from others. At the *Glu-A1* locus, *Glu-A1a* has been reported to exhibit better quality than *Glu-A1c* in some studies, including our study. At the *Glu-D1* locus, *Glu-D1d* (5 + 10) has been reported to have better dough and bread-making qualities than *Glu-D1a* (2 + 12) (Hsam *et al.* 2001; Zheng *et al.* 2009) and *Glu-D1f* (2.2 + 12) (Payne 1983; Zeng *et al.* 2001). Moreover, *Glu-D1h* (5 + 12) has been shown to exhibit better dough quality than *Glu-D1d* (Pena *et al.* 1995; Wang *et al.* 2004). In our study, *Glu-D1d* was associated with higher MPT, MPV, MPI and 8MW values than *Glu-D1h*, and the results were significant, which perhaps was caused by the smaller samples with *Glu-D1h* allele in the RIL population. Compared with *Glu-D1new1* (2.2 + 10), *Glu-D1f* had higher MPT, MPW and MPI values, but these differences were not significant. In this population, the rank order of effect of the *Glu-D1* loci on dough quality was  $Glu-D1d \geq Glu-D1h > Glu-D1f \geq Glu-D1new1$ .

Waxy proteins are responsible for amylose synthesis in the endosperm. The effects of waxy proteins on starch properties, especially pasting properties and bread-making quality, have been extensively studied using different flours. Farinograph water absorption was significantly higher for waxy wheat than for nonwaxy wheat (Guo *et al.* 2003; Takata

*et al.* 2005). In our study, the amount of water added in the Mixograph was in accordance with the Farinograph water absorption data. The wild type (a/a/a) showed higher MPT and MPI values than whole waxy wheat (b/b/b), which is consistent with results from a study by Zhai *et al.* (2008). The results were potentially caused by the presence of more damaged starch granules in the waxy wheat than in the nonwaxy wheat. When one waxy locus was null, there were almost no significant differences for dough properties, except (b/a/a) with two waxy locus deletions. However, MPV and 8MW levels of partial waxy wheat were higher than those of the wild type, indicating a good mixing tolerance and resistance.

### Interactions among glutenin and *Wx* loci

The effects of the lack of a single locus among the glutenin and *wx* loci on wheat quality have previously been studied (Takata *et al.* 2005; Zhai *et al.* 2008; Zhang *et al.* 2009a; Yamamori 2009; Debiton *et al.* 2010). Because glutenin and waxy loci are segregated in the Nuomai1/Gaocheng8901 RIL population, we had the unique opportunity to investigate the effects of their pairwise interaction and the interaction of HMW-GS and *Wx* subunit combinations on Mixograph properties under the same genetic background. Interactions between *Glu-1* and *Wx-1* loci significantly ( $P < 0.05$ ) affected some Mixograph properties. The combinations *Glu-A1a/Wx-B1a*, *Glu-A1c/Wx-B1a*, *Glu-A1c/Wx-D1a*, *Glu-D1f/Wx-A1a*, *Glu-D1d/Wx-B1a*, *Glu-D1f/Wx-B1a* and *Glu-D1new1/Wx-B1a* were associated with lower ( $P < 0.05$ ) MPV values than the other allelic combinations. In addition, interactions between *Glu-D1d* and three *Wx-1* loci significantly affected the Mixograph characteristics. Some alleles at the *Glu-1* and *Wx-1* loci were not significantly different from each other in the Mixograph characteristics in our population. When inferior alleles at the *Glu-1* loci were present, interactions between *Glu-1* loci and the deletions of *Wx-1* loci were significantly different from other allelic combinations for some Mixograph traits, such as MPV, MPW, and 8MW. Analysis of this interaction indicates the importance of screening for both *Glu-1* and *Wx-1* alleles in wheat breeding programmes, especially special noodle wheat breeding.

Presence of good or inferior alleles at *Glu-1* loci might be associated with lack or presence of particular alleles at *Wx-1* loci. So the *Glu-1* and *Wx-1* loci might compensate for each other in terms of their effect on wheat quality. For example, *Glu-A1c/Wx-B1b* and *Glu-D1f/Wx-B1b* showed significantly higher MPV values than *Glu-A1c/Wx-B1a* and *Glu-D1f/Wx-B1a*. *Glu-D1d/Wx-B1a* and *Glu-D1d/Wx-D1a* showed significantly higher MPT than *Glu-D1d/Wx-B1b* and *Glu-D1d/Wx-D1b*, respectively. Therefore, careful selection of glutenin and waxy allele composition may be a good way to obtain favourable results in special wheat breeding programmes.

### QTL analysis for dough-mixing strength

One major QTL cluster on chromosome 1D that affected three dough-mixing properties (MPT, MPI and 8MW) was

mapped to the HMW glutenin *Glu-D1* loci, which is consistent with previous research (Huang *et al.* 2006; Elangovan *et al.* 2008; Wu *et al.* 2008; Zhang *et al.* 2009b; Kerfal *et al.* 2010; Tsilo *et al.* 2011). Other QTL clusters, on chromosomes 1A, 1B, 3B, 4A, 4D and 6A, that influence dough-mixing characteristics were also found. Among them, the one minor QTL cluster on chromosome 1A that controls MPT, MPV and MPI was mainly located between wPt9757 and *Glu-A1* and was stable in different environments, confirming that its contribution to dough quality is inferior to that of *Glu-1D*. Tsilo *et al.* (2011) also found QTL for MPV and MPW at XwPt9429–XwPt9757 and Xgwm357–XwPt9757, respectively. Three QTL associated with *Glu-B1* in the same region were detected for MPT, MPI and 8MW. These results indicated that *Glu-A1* and *Glu-B1* also contribute to dough-mixing quality and underscore the importance of *Glu-1* loci on dough-mixing quality.

The QTL identified between wPt9432 and wPt9510 on chromosome 3B mainly affected the MPT, MPI and 8MW, and this location seemed to control the protein content based on the consensus map of Somers *et al.* (2004). The wPt5870 marker on chromosome 3B revealed that the QTL mainly control MPW and 8MW.

We also found that QTL for MPT, MPI and 8MW were found in the same region on chromosome 6A and were stable in different environments. However, this QTL cluster only had a minor effect on these properties. This is the first report on the effects of chromosome 6A on dough-mixing quality.

New QTL related to starch quality were detected on chromosome 4A with flanking markers wPt664948–*Wx-B1* in three environments and controlled the Mixograph peak value. Its additive effect comes from Nuomai1. This waxy protein is not expressed at *Wx-B1* but contributes to the dough Mixograph peak value, perhaps by decreasing the amylose content and changing the ratio of amylose and amylopectin. This indirectly affected mixing properties by adjusting the starch and protein content. Thus, the contribution of *Wx-B1* to dough quality is larger than that of the other two loci. Therefore, QTL for traits could be completed by analysing different genetic populations.

Our results indicated that the interactions between HMW-GSs and waxy proteins play important roles in determining dough-mixing properties at the protein subunit and QTL levels. MPV was mainly affected by interactions between *Glu-1* and *Wx-1* loci and QTL affecting it were located around *Wx-B1*. Variation at *Wx-1* loci played a minor role in variation in dough-mixing properties, but *Glu-A1* and *Glu-D1* loci were major contributors to dough-mixing properties. There was some indication that *Glu-A1c*, the null allele, was inferior in dough strength compared to *Glu-A1a*. The beneficial effects of *Glu-D1d* over *Glu-D1f* and of *Glu-D1h* over *Glu-D1new1* for dough-mixing properties were confirmed for the first time using the RIL population. Interactions between different glutenin and waxy alleles affected the different Mixograph mixing characteristics. Eight major QTL in three environments were found, including the stable QTL

*QMPT-1D.1*, *QMPI-1D.1*, *QMPI-1D.2*, *Q8MW-1D.1* and *Q8MW-1D.2*, which can be used as molecular markers for selection in breeding. Fourteen QTL for Mixograph parameters were found to be associated with genomic regions other than *Glu-1* loci and were mapped in one case in the same region as the QTL for different mixing characteristics. Because the RIL population was derived from two special cultivars, we hope that the QTL identified in this study will be useful in accumulating beneficial alleles through marker-assisted selection, thereby facilitating the development of high-quality wheat cultivars. Moreover, the effects of the new QTL identified in this study would be validated by developing new breeding populations derived from the selected RILs.

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