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Development of introgression lines with 18 alleles of glutenin subunits and evaluation of the effects of various alleles on quality related traits in wheat (*Triticum aestivum* L.)

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ABSTRACT

In this report, we present a set of 104 ILs with 18 alleles for five glutenin loci. They were developed from crossing and backcrossing 64 varieties as donor parents to Yanzhan 1 as recurrent parent. The effects of the 18 alleles on nine dough quality parameters were evaluated in a similar background using these lines. The results showed that *Glu-A1a* produced the highest SDS-sedimentation volume (Ssd), midline time x=8 width (MTxW), mixing tolerance (MT) and the lowest weakening slope (WS). At the *Glu-B1* locus, *Glu-B1f* produced the highest values for all quality parameters but WS. At the *Glu-D1* locus, *Glu-D1d* was the best for Ssd, grain hardness (GH), midline peak width (MPW), MTxW and MT. The positive effects of *Glu-B1f* on GH and *Glu-B3b* on Ssd were mainly from the effect of GPC. Overall, 5 interactive loci and 13 interactive alleles were found to be significant. No negative interaction between high quality glutenin alleles was detected. The preferred allele combinations for breeding were recommended based on the additive and interactive effects. Our results suggest that the ILs with multiple alleles are ideal genomic stocks for evaluating the effects of alleles on some traits and for pyramiding favorable alleles in breeding wheat varieties.

1. Introduction

Wheat (*Triticum aestivum* L.) storage protein is composed of gliadins and glutenins, which are the main components of gluten, and are the important factors influencing the bread-making quality. Glutenins generally fall into two basic classes based on the differences in molecular weight of their subunits: high molecular weight glutenins (HMW-GSs) and low molecular weight glutenins (LMW-GSs). Genes encoding the HMW-GSs are located at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arms of chromosomes 1A, 1B and 1D, while those encoding the LMW-GSs, *Glu-A3*, *Glu-B3* and *Glu-D3*, are located on the short arms of the group 1 chromosomes (Gupta and Shepherd, 1990; Jackson et al., 1983). Each *Glu-1* locus has two genes encoding two glutenin subunits, x-type and y-type (Payne et al., 1981).

Results from previous studies on the relationship between glutenin subunits and end-use quality have confirmed that HMW-GS are highly correlated with bread baking quality (Payne et al., 1979, 1987, 1987; Gupta et al., 1989; Carrillo et al., 1990; Sontag-Strohm et al., 1996). LMW-GS and gliadins were also found to influence the bread-making quality (Payne et al., 1987) but with inconsistent results. This is mostly due to the use of different genetic materials with various genetic backgrounds in different studies. For example, when measuring the effect of the subunits 2* on SDS-sedimentation volume, a value of =1 was observed from using the British-grown wheat varieties (Payne et al., 1987), whereas a value of >1 was found by Mao et al. (1995) using different wheat varieties, and a value of <1 was obtained by Liu et al. (2005) using 251cultivars and advanced lines.

Bread-making quality can be better explained as a result of additive and interactive effects of high- and low-molecular weight glutenin subunits (Gupta et al., 1994). The interactive effects between glutenin loci have been reported previously (Carrillo et al.,

Abbreviation: AACC, American Association of Cereal Chemists; GH, grain hardness; GPC, grain protein content; HMW-GS, high molecular weight glutenin subunit; IL, introgression line; LMW-GS, low molecular weight glutenin subunit; MPT, midline peak time; MPV, midline peak value; MPW, midline peak width; MT, mixing tolerance; MTxW, midline time x=8 width; NIL, near-isogenic line; NIRS, near-infrared reflectance spectroscopy; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; Ssd, SDS-sedimentation volume; WS, weakening slope.

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1990; Gupta et al., 1994; Eagles et al., 2002; Liu et al., 2005). Most of these results gave only the interactive effect between specific pairs of loci, and the significant interactive effects between individual alleles at different loci are not clear. Moreover, the noise of genetic background in different cultivars can also confound the estimate of the exact interactive effects. As a result, the theoretical basis for developing varieties with preferred subunit combinations is lacking.

In contrast NILs and ILs carrying different alleles of glutenin genes developed in a similar genetic background are ideal genetic stocks to evaluate allelic effects on wheat quality. Based on three NILs developed using the same donor parent, Deng et al. (2005) demonstrated that the contribution of subunit pair 14 + 15 (*Glu-B1h*) on bread-making quality was higher than that of 7 + 9 (*Glu-B1c*), and subunit pair 5 + 10 (*Glu-D1d*) was higher than subunit 10. The allele of *Glu-D1d* on sedimentation value was higher than that of *Glu-D1a* by using the NILs carrying *Glu-D1d* and *Glu-D1a* (Zhang et al., 2003). However, the influence of other known alleles on wheat quality and the interaction among these alleles have not been evaluated using NILs or ILs due to the lack of the availability of suitable materials. The main objectives of the present study were to develop ILs with multiple alleles for glutenin subunits and to evaluate the effects of multiple alleles of HMW-GSs and LMW-GSs on quality related traits and to estimate the interactive effects among these alleles.

2. Experimental

2.1. Plant materials

The ILs were generated by using Yanzhan 1, a Chinese commercial cultivar, as the recurrent parent. It was crossed and backcrossed with 64 varieties, including varieties bred in China, landraces and introduced varieties. Among 104 lines selected, eight were backcrossed twice, 80 three times and 16 four times to the recurrent parent. These ILs and their parents were grown in the experimental fields at the Luoyang Agricultural Academy of Science, Henan, China in 2004–2005 and 2005–2006. Each line was grown in a 2 m long double-row plot in each environment, and the recurrent parent as a check was planted in five replications at each environment. Field management was in accordance with local practice and no pesticides were used.

2.2. HMW-GS and LMW-GS analysis

The method used for extracting gliadin (for evaluating the alleles of the *Glu-B3* locus) and glutenin (for evaluating the alleles of *Glu-A1*, *Glu-B1*, *Glu-D1* and *Glu-A3* loci) were based on Singh et al. (1991) with slight modifications (Liu et al., 2005). These two fractions were then prepared separately for SDS-PAGE analysis according to Singh et al. (1991). Ten seeds were analyzed for each line to check for homozygosity.

The HMW-GSs were identified according to Payne and Lawrence (1983), and the LMW-GSs were identified according to Singh et al. (1991) and Jackson et al. (1996). The presence of the *Glu-B3j* (*null*) allele was an indication of the 1B.1R translocation (Gupta and Shepherd, 1992). The alleles at *Glu-D3* were not identified in this procedure due to difficulty in identifying the different forms.

2.3. Phenotypic assessments

SDS sedimentation volumes (Ssd) were determined using the methods modified from Axford et al. (1979) and Preston et al. (1982). SDS-lactic acid reagent was prepared by dissolving 20 g of SDS in 1 L of water and adding 20 ml of stock diluted lactic acid solution (one part of lactic acid plus eight parts of water by volume). Briefly, a 2-g whole meal flour was placed in a 35-ml graduated cylinder, 16.7 ml water containing methylene blue was added, and the cylinder was vortexed for 5 min. Then 16.7 ml SDS-lactic acid reagent was added and cylinders were mixed for 5 min. Finally, the cylinders were placed vertically and the sedimentation volume was determined 5 min later. This trait was evaluated for materials grown in both of the environments.

The grain protein content (GPC) and hardness (GH) were measured on a 2-g sample of whole-meal flour by near-infrared reflectance spectroscopy (NIRS) on a Perten DA7200 instrument and expressed on a 14% moisture basis. The measurements were calibrated according to known samples.

A 10-g Mixograph was performed to record midline peak time (MPT, min), midline peak value (MPV, %), midline peak width (MPW, %), midline time X=8 width (MTxW, %), mixing tolerance (MT, min) and weakening slope (WS, %), according to AACC method 54-40A (AACC, 1983).

2.4. Statistical analysis

Pearson's correlation coefficients between traits were calculated using the Correlate of SPSS 15.0 software. LSD multiple comparisons were carried out to examine the quality effect of individual subunit/allele.

The interactions between loci were estimated using the 2-way interactions in GENSTAT based on the methods of Eagles et al. (2002). The interaction between any two alleles (S_{ij}) was estimated by the special combining ability between the concerned alleles, according to the following formula: $S_{ij}=y_j-\hat{y}_i-\hat{y}_j+\hat{y}..$, where y_{ij} was the mean value of allele i and j combinations; $\hat{y}_{i.}$ and $\hat{y}_{j.}$ were the mean value of the combinations of allele i and j with other alleles, and $\hat{y}..$ was the mean of the population.

The regression analysis was performed based on Branlard and Dardevet (1985a). The values of the traits which have linear relationships with GPC can be corrected by eliminating the effect arising from the GPC according to the equation: $y'=y-b\,(x-10.2)$, where $b=\mathrm{cov}(y, x)/\mathrm{var}(x)$, y' is the corrected value, y is the given value, x is the given protein content, and the protein content of the reference (10.2%) was the minimum value found for our genotypes. When test values were not related in a linear manner to protein content, corrections were not made.

3. Results

3.1. Development of ILs for five of the glutenin loci

Yanzhan 1 was crossed with the 64 donor parents, backcrossed with Yanzhan 1 at least twice, which was then followed by selfing. One hundred and four ILs which showed similar phenotypes with their recurrent parent Yanzhan 1 were selected. The allele of HMW-GSs and LMW-GSs of the recurrent parent and their 104 IL were detected by using SDS-PAGE analysis. The results showed that the recurrent parent Yanzhan 1 had the following HMW-GS composition: N, 14 + 15, 4 + 12, encoded at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively, while LMW-GSs had the *Glu-A3b* and *Glu-B3d* alleles. Altogether, eighteen alleles (sample number >4) for glutenin subunits were detected among the 104 ILs studied. They included three for *Glu-A1*, four for *Glu-B1*, three for *Glu-D1*, three for *Glu-A3*, and five for *Glu-B3* (Table 1). The combinations among these alleles resulted in 38 different genotypes. These ILs were subsequently subjected to further analyses as described below.