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ORIGINAL PAPER

Using near-isogenic lines of common wheat (*Triticum aestivum* L.) to determine the relationship between GlutoPeak parameters and quality characteristics

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Abstract

The GlutoPeak is a new instrument used to measure the rheological properties of wheat flour dough. To determine the role of GlutoPeak parameters in assessing wheat quality, near-isogenic lines (NILs) of three wheat varieties with different gluten strengths were used in this study. The availability of GlutoPeak parameters was determined by comparing the consistency of differences in GlutoPeak parameters with the differences in the genetic effects of NILs and the differences in the conventional quality parameters caused by the genetic effects of those NILs. The results showed that only the difference in peak maximum time was identical to the genetic differences and conventional quality parameters within each of these NIL sets. The conventional quality parameters examined were gluten index, Zeleny sedimentation, development time, stability, breakdown time, maximum resistance, extensibility and energy area. Higher gluten strength corresponded to larger peak maximum time. These results provide valuable information concerning the application of the GlutoPeak to the improvement of wheat quality.

Keywords Wheat quality · Quality characteristics · GlutoPeak · Conventional rheological parameters · NILs

Introduction

End-use quality improvement is a major challenge in wheat breeding (*Triticum aestivum* L.) [9]. In wheat breeding programmes, the quality attributes of wheat varieties should be precisely evaluated to determine which parents to cross, and the obtained progeny must be evaluated to select lines that have desirable traits [21]. Compared with other flour quality testing techniques, rheometry is considered a better technique to predict final product quality [5, 10]. The rheological properties of wheat flour dough can also be accurately measured by the farinograph and extensograph (Brabender Gmb Hand Co KG, Duisburg, Germany), which are widely used in wheat breeding [6, 9]. However, these machines require a large amount of time, a large sample amount and considerable labour [3, 8, 14, 15]. The GlutoPeak (Brabender GmbH and Co. KG, Duisburg, Germany) is a new instrument that measures the rheological properties of wheat flour dough. The GlutoPeak has the advantages of small sample amount (< 10 g), rapid analysis (< 10 min), easy operation and excellent reproducibility [4, 16], and it is thus suitable for wheat breeding programmes [14, 15, 20]. Studies utilizing various materials and methods for this instrument have been carried out by many researchers [3, 4, 8, 13–16, 20]. However, a consistent relationship between GlutoPeak parameters and quality characteristicshas not yet been determined. The role of GlutoPeak parameters in assessing wheat quality is still a debated problem, which a more precise study is required to solve.

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Wheat flour quality is a complex trait controlled by many genes [1]. Gliadin and glutenin (high-molecular-weight and low-molecular-weight glutenin subunits), which are two of the main factors that determine dough cohesiveness and elasticity, are controlled by genes at twelve complex loci [18]. In addition, the influence of environmental conditions on quality traits is also complex. Due to differences in genetic background, variation among ecotypes, developmental stages and maturation periods exist among varieties [21]. The baseline and magnitude of variation in quality are different when varieties are grown in the same place in different years or in different places in the same year [26]. Therefore, it is difficult to exactly analyse the effect of a given gene [12, 25, 26]. However, the problems mentioned above can be easily solved if near-isogenic lines (NILs) are used [1, 22, 23]. First, any differences in quality characteristics observed within a set of NILs are due to a qualitative trait controlled by one allelic gene. Thus, the analysis of genetic effects becomes simpler and clearer. Second, because NILs have the same genetic background, the norm of reaction is consistent when environmental conditions change. Thus, the differences in quality characteristics within a set of NILs are unaffected by environmental conditions [1, 23].

NILs of the varieties Long97-586 (weak gluten) with subunit 7 and subunits 7+8 at the *Glu-B1* locus, Longfumai3 (medium gluten) with null and subunit 1 at the *Glu-A1* locus, and Xiaobingmai33 (strong gluten) with subunits 2+12 and subunits 5+10 at the *Glu-D1* locus were used in this study. The aim of the present study was to determine the role of GlutoPeak parameters in assessing wheat quality by comparing the differences in GlutoPeak parameters with the differences in genetic effect and conventional rheological parameters within each of these NIL sets. The results are clear, exact, and simple to analyse and provide useful information for the application of the GlutoPeak in breeding programmes.

Materials and methods

Plant materials and field experiment design

The NILs of the three wheat varieties used in this study were created by Prof Yanbin Zhang, Crop Breeding Institute, Heilongjiang Academy of Agricultural Sciences [12, 25, 26]. The NILs of Long97-586, with subunit 7 and subunits 7+8, were produced by five consecutive backcrosses to Long97-586 [25]. The final selected lines, Long 03-327 (Long97-586 with subunit 7) and Long 03-326 (Long97-586 with subunits 7+8), were obtained in 2003. The NILs of Longfumai3, with null and subunit 1, were produced by five consecutive backcrosses to Longfumai3 [26]. The final selected lines, Long 02-190 (Longfumai3 with null) and Long 02-192 (Longfumai3 with subunit 1), were obtained in 2002. The NILs of Xiaobingmai33 with subunits 2+12 and subunits 5+10, were produced by six consecutive backcrosses to Xiaobingmai33 [12]. The final selected lines, Long 01-496 (Xiaobingmai33 with subunits 2+12) and Long 01-497 (Xiaobingmai33 with subunits 5+10), were obtained in 2001. Each NIL consisted of sister lines derived from the same BC-5 or BC-6 plant followed by self-separation. Table 1 shows the high-molecular-weight glutenin subunits (HMW-GS) composition in the *Glu-1* allele of the NILs of each variety. The NILs were flat-planted in the experimental field of the Heilongjiang Academy of Agricultural Sciences in 2012. The field experiments were designed using a two-column contrast arrangement method with four replicates. To ensure comparable cultivation conditions, each plot group was fertilized by the same hole of the seeding machine and irrigated by the same nozzle head of the hose reel irrigator.

Methods

Grain sample were milled on a Quadrumat® Senior mill (Brabender Co., Germany). Measures of kernel protein content, flour protein content, dry gluten content, gluten index, Zeleny sedimentation values, and farinograph and

Table 1 The high-molecular-weight glutenin subunits (HMW-GS) alleles at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci in the near-isogenic lines (NILs) of each variety

Material	HMWG subunits			Classification
	Glu-A1	*Glu-B1*	*Glu-D1*	
Long97-586 with subunit 7	1	7	2+12	Weak gluten
Long97-586 with subunits 7+8	1	**7+8**	2+12	
Longfumai3 with null	Null	7+8	5+10	Medium gluten
Longfumai3 with subunit 1	**1**	7+8	5+10	
Xiaobingmai33 with subunits 2+12	2*	7+8	2+12	Strong gluten
Xiaobingmai33 with subunits 5+10	2*	7+8	**5+10**	

Bold indicates introduced high-molecular-weight glutenin subunit extensograph parameters were performed by the methods reported in Song et al. [21]. Gluten quality by GlutoPeak was measured at Brabender Co., Germany, in December 2012. 9 g of flour was dispersed in 1/3 mol sodium chloride solution (10 mL) into a sample cup. The speed was set at 3000 rpm for the rotating paddle. Temperature was controlled at 36 °C by circulating water through the jacketed sample cup. All the statistical analyses of the data were performed by SPSS software 17.0.

Results

The kernel protein, flour protein, dry gluten and Zeleny sedimentation

Previous research has shown that the protein content [11, 12, 19, 24, 25] and dry gluten content [12, 21, 25, 26] are basically identical within each set of NILs. As shown in Table 2, no significant differences in kernel protein content, flour protein content or dry gluten content were found within a set of NILs ($P>0.05$) (Table 2). Gluten index is an important criterion to define gluten quality [2, 17]. Among the three sets of NILs, the gluten index of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 9.0% ($P=0.486$), 10.4% ($P=0.258$) and 2.3% ($P=0.024$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. The Zeleny sedimentation of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 34.7% ($P=0.015$), 5.4% ($P=0.015$) and 10.8% ($P=0.029$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. These results of the gluten index and Zeleny sedimentation basically agree with those of previous studies [12, 25, 26].

Farinograph and extensograph parameters

As shown in Table 2, the development times of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 50.8% ($P=0.031$), 65.9% ($P=0.066$) and 51.3% ($P=0.060$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12 within these NIL sets. The stability of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 30.5% ($P=0.149$), 33.1% ($P=0.018$) and 65.6% ($P=0.002$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. The breakdown time of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 40.9% ($P=0.032$), 31.9% ($P=0.038$) and 49.8% ($P=0.003$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. The difference in quality characteristics determined by these rheological parameters within each of these NIL sets was identical to that found in previous research [12,

Table 2 Results of quality parameter evaluation in three sets of near-isogenic lines (NILs)

Quality parameter	Long97-586				Longfumai3				Xiaobingmai33			
	7	7+8	Increase	*P* value	Null	1	Increase	*P* value	2+12	5+10	Increase	*P* value
Kernel protein (%)	14.4	14.7	1.9	0.168	15.4	15.2	−1.5	0.136	15.9	16.2	1.8	0.261
Flour protein (%)	12.2	12.3	1.2	0.156	12.0	12.3	2.4	0.176	14.2	14.5	1.8	0.346
Dry gluten (%)	10.1	10.6	4.3	0.123	9.6	9.6	−0.1	0.905	12.0	11.6	−3.3	0.157
Gluten index (%)	69.4	75.6	9.0	0.486	80.9	89.3	10.4	0.258	96.9	99.1	2.3	0.024
Zeleny sedimentation (mL)	18.0	24.3	34.7	0.015	41.8	44.0	5.4	0.015	50.8	56.3	10.8	0.029
Water absorption (%)	54.0	55.3	2.4	0.002	53.8	53.4	−0.7	0.317	54.8	58.7	7.1	0.508
Development time (min)	1.6	2.4	50.8	0.031	3.1	5.1	65.9	0.066	9.4	14.2	51.3	0.060
Stability (min)	1.5	1.9	30.5	0.149	6.2	8.3	33.1	0.018	17.2	28.5	65.6	0.002
Breakdown time (min)	2.2	3.1	40.9	0.032	6.8	9.0	31.9	0.038	18.4	27.5	49.8	0.003
Maximum resistance (BU)	76.7	118.0	53.9	0.005	266.5	336.3	26.2	0.084	954.3	1420.0	48.8	0.001
Extensibility (mm)	211.0	205.3	−2.7	0.375	153.8	151.3	−1.6	0.650	171.5	129.5	−24.5	0.018
Energy area (cm^2)	23.7	35.7	50.7	0.010	56.3	67.0	19.1	0.105	200.8	213.8	6.5	0.358
Lift off time (s)	0.0	0.0	–	–	85.1	101.6	19.4	0.070	79.1	147.1	85.9	0.002
Peak maximum time (s)	57.1	66.9	17.1	0.033	123.4	152.8	23.8	0.015	144.0	219.8	52.6	0.001
Maximum torque (BE)	34.8	41.9	20.5	0.024	28.6	32.3	12.7	0.006	46.6	43.4	−7.0	0.016
Torque before maximum (BE)	23.8	15.6	−34.2	0.236	13.6	29.9	119.3	0.025	43.9	39.6	−9.7	0.127
Torque after maximum (BE)	29.6	32.5	9.7	0.022	24.5	29.0	18.4	0.051	42.5	39.9	−6.2	0.050

The data are averaged from four replicates in 2012. The *P* value was calculated by the *t* test on binate data of the NILs

25, 26]. The water absorption of Long97-586 with subunits 7+8 and Xiaobingmai33 with subunits 5+10 were 2.4% ($P=0.002$) and 7.1% ($P=0.508$) higher than those of Long97-586 with subunit 7 and Xiaobingmai33 with subunits 2+12; the water absorption of Longfumai3 with subunit 1 was 0.7% ($P=0.317$) lower than Longfumai3 with null. The results of the water absorption assay basically agree with previous reports between the NILs of Long97-586 [25]; however, the results of the water absorption assay disagree with previous reports regarding the NILs of Longfumai3 and Xiaobingmai33 [12, 26]. The cause of this difference will require further study.

Within these NIL sets, the maximum resistance of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 53.9% ($P=0.005$), 26.2% ($P=0.084$) and 48.8% ($P=0.001$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. The energy areas of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 50.7% ($P=0.010$), 19.1% ($P=0.105$) and 6.5% ($P=0.358$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. The extensibility of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 2.7% ($P=0.375$), 1.6% ($P=0.650$) and 24.5% ($P=0.018$) lower than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12. Among the three sets of NILs, higher gluten strength is correlated with lower extensibility. The quality characteristics determined by the maximum resistance, extensibility and energy area measured by extensograph is identical to that determined by the development time, stability and breakdown time measured by farinograph across the three sets of NILs in this study

GlutoPeak parameters

Of the GlutoPeak parameters (Table 2), the peak maximum times of Long97-586 with subunits 7+8, Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 17.1% ($P=0.033$), 23.8% ($P=0.015$) and 52.6% ($P=0.001$) higher than those of Long97-586 with subunit 7, Longfumai3 with null and Xiaobingmai33 with subunits 2+12 within these NIL sets. The index of lift off time was zero in the NILs of the weak gluten wheat variety Long97-586. The lift off times of Longfumai3 with subunit 1 and Xiaobingmai33 with subunits 5+10 were 19.4% ($P=0.070$) and 85.9% ($P=0.002$) higher than those of Longfumai3 with null and Xiaobingmai33 with subunits 2+12. The maximum torque values of Long97-586 with subunits 7+8 and Longfumai3 with subunit 1 were 20.5% ($P=0.024$) and 12.7% ($P=0.006$) higher than those of Long97-586 with subunit 7 and Longfumai3 with null, but that of Xiaobingmai33 with subunits 5+10 was 7.0% ($P=0.016$) lower than Xiaobingmai33 with subunits 2+12. The torque before maximum values of Long97-586 with subunits 7+8 and Xiaobingmai33 with subunits 5+10 were 34.2% ($P=0.236$) and 9.7% ($P=0.127$) lower than Long97-586 with subunit 7 and Xiaobingmai33 with subunits 2+12, but that of Longfumai3 with subunit 1 was 119.3% ($P=0.025$) higher than Longfumai3 with null. The torque after maximum values of Long97-586 with subunits 7+8 and Longfumai3 with subunit 1 were 9.7% ($P=0.022$) and 18.4% ($P=0.051$) higher than those of Long97-586 with subunit 7 and Longfumai3 with null, but that of Xiaobingmai33 with subunits 5+10 was 6.2% ($P=0.050$) lower than Xiaobingmai33 with subunits 2+12.

Discussion

NILs are the best materials for evaluating the availability of quality parameters

Among varieties, the genes controlling the same quality traits are different in number and type. In addition to the differences caused by genetic background, individual quality traits are easily affected by environmental conditions. When one or more quality parameters are used to characterize varieties, stable and consistent results are difficult to obtain [7, 15]. However, the differences in certain quality traits within a set of NILs is determined by one or two genes. Thus, NILs have a qualitative character that is easy to analyse. Because NILs have the same genetic background, the ecotype, developmental stage and maturation period are identical within each set of NILs. Therefore, the influence of environmental conditions on NILs is equal, and the interference from the genetic background and environmental conditions can be eliminated in an analysis of the genetic effects of HMW-GS by using NILs [1, 12, 21, 25, 26].

Three sets of NILs differing at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci were used in this study. The differences in quality characteristics within each set of NILs caused by a different gene at each locus were obvious and stable. Overall, every quality parameter measured by the glutomatic system, farinograph or extensograph could easily distinguish the quality characteristics within each set of NILs, and most importantly, the difference within NILs was unaffected by environmental conditions [21]. Therefore, GlutoPeak parameters that can precisely distinguish quality characteristics should accord with the genetic differences within the NILs. We could precisely analyse the effect of genes on quality by comparing the NILs via the parameters measured by the analytical equipment. Conversely, we could evaluate the availability of quality parameters by using NILs of known genetic effect. Compared with studies using varieties, studies using NILs save time, and the data analysis is simple and precise. We can do less work, and the results will be better. To precisely analyse the availability of GlutoPeak parameters, three sets of NILs with different gluten strengths, including weak, medium and strong gluten, were used in this study. The differences in gluten strength among the three varieties were obvious. The rankings were as follows: Xiaobingmai33 > Longfumai3 > Long97-586. Quality parameters, which can precisely evaluate gluten strength, should be able to distinguish the quality differences among these three varieties.

Quality characteristics, as determined by the gluten index, Zeleny sedimentation, development time, stability, breakdown time, the maximum resistance, energy area, could not only precisely distinguish the quality differences within each set of NILs but also rank all materials. The rankings were as follows: Xiaobingmai33 with subunits 5+10 > Xiaobingmai33 with subunits 2+12 > Longfumai3 with subunit 1 > Longfumai3 with null > Long97-586 with subunits 7+8 > Long97-586 with subunit 7. The above results suggest that the differences in genetic effect within each of these NIL sets are significant and invariable. These materials could be used to precisely evaluate the availability of individual GlutoPeak parameters in quality assessment.

The availability of individual GlutoPeak parameters in assessing quality characteristics

The GlutoPeak is a new instrument that measures the rheological properties of wheat flour dough and is suitable for wheat breeding programmes to assess the quality characteristics of varieties [14, 15]. Several studies regarding the GlutoPeak test have been carried out in recent years. Marti et al. [15] investigated the correlations between GlutoPeak parameters and conventional rheological parameters measured by farinograph, alveograph and extensograph by using 120 commercial wheat flours. The results showed that the energy value and the maximum torque were the most significant GlutoPeak parameters in the prediction of conventional parameters. Sissons [20] reported that the most discriminating parameter is peak maximum time, and it correlated strongly with mixograph parameters and gluten index but not with SDS sedimentation by using durum wheat semolina. In this study, the GlutoPeak parameters were investigated by using three sets of NILs with different quality characteristics as determined by conventional parameters including gluten index, Zeleny sedimentation, farinograph and extensograph parameters. The results showed that only the peak maximum time, out of all GlutoPeak parameters, displayed a consistent performance across the three sets of NILs. Higher gluten strength was correlated with larger peak maximum time. In addition, the quality characteristics determined by the peak maximum times were identical to those determined by the gluten index, Zeleny sedimentation, development time, stability, breakdown time, maximum resistance, extensibility, energy area. The quality characteristics difference within each set of NILs could be distinguished accurately by the peak maximum time. However, this parameter could not accurately distinguish between the gluten strength values of Xiaobingmai33 with subunits 2+12 and Longfumai3 with subunit 1, which were in different NIL sets. The lift off time was zero in the NILs of the weak gluten variety Long97-586. However, in the Xiaobingmai33 and Longfumai3 NILs, the lift off time performance was identical to the conventional parameters that determine quality characteristics. The role of lift off time in assessing quality traits should be further investigated. Marti et al. [14, 15] reported that the maximum torque was the most significant parameter for the prediction of conventional parameters. However, Fu et al. [8] recently found that the maximum torque was independent of dough strength as measured by extensograph. In this study, none of the torque parameters, including maximum torque, torque before maximum, and torque after maximum, displayed consistent performance across the three sets of NILs (Table 2). The quality characteristics differences between NILs were not accurately distinguished by the torque parameters. These results suggested that the relationship between torque and quality characteristics is complex.

Investigation of an improved method for GlutoPeak parameter use

The GlutoPeak represents a new rheometry method to assess wheat quality characteristics. Some improved methods of using GlutoPeak parameters in quality assessment have been proposed. Two parameters, area under the peak and aggregation time, were added as quality parameters of wheat flour by Marti et al. [15] and Fu et al. [8]. In addition, energy value, including aggregation energy, energy to maximum torque, energy after maximum torque, were added by Malegori et al. [13]. We also believe that the energy under the peak is related to the gluten strength. This parameter should be useful in quality assessment. However, the role of this parameter in assessing gluten strength within the NILs should be further investigated in cooperation with the manufacturer.

Conclusions

According to our results, only the peak maximum time out of all GlutoPeak parameters is identical to the gluten index, Zeleny sedimentation, development time, stability, breakdown time, the maximum resistance, energy area within each of these NIL sets. The differences in peak maximum time are identical to the differences in gluten strength within the NILs, which are caused by differences in the genes at each locus. In addition, the peak maximum time is identical to the conventional parameters within the NILs. Thus, the peak maximum time represents the availability parameter of GlutoPeak to distinguish quality characteristics. Higher gluten strength is correlated with larger peak maximum time.

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